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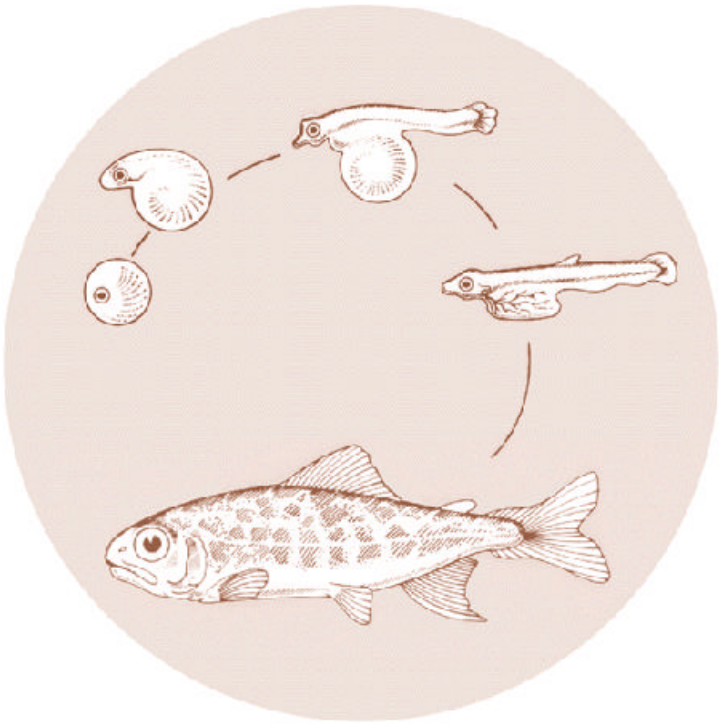
PROCEEDINGS: WORKSHOP ON VIRAL DISEASES OF SALMONID FISHES IN THE COLUMBIA RIVER BASIN

Portland, Oregon
October 7/8, 1983



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P.O. Box 3621
905 N.E. 11th Avenue
Portland, OR 97208-3621

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October 7-8, 1982

Proceedings, April 1983

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Edited by

JoAnn C. Leong
Department of Microbiology
Oregon State University
Corvallis, Oregon

Theresa Y. Barila
Bonneville Power Administration
P.O. Box 3621
Portland, Oregon

Published by

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P.O. Box 3621
Portland, Oregon 97208

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A map of Columbia River Basin Hatcheries will be published as a supplement to the proceedings. The map will illustrate the incidence of IHNV within the Columbia River Basin.

Preface

The objective of the "Workshop on Viral Diseases of Salmonid Fishes in the Columbia River Basin" was to summarize the status of current research activity, and to discuss and define research needs concerning fish viruses affecting salmonids within Columbia River Basin.

Bonneville Power Administration's (BPA) role in efforts in fish diseases and more generically the protection, mitigation, and enhancement of Columbia River salmon and steelhead populations, has recently been expanded through the passage by Congress of the Pacific Northwest Electric Power Planning and Conservation Act (Regional Act>, Pub. L. 96-501. Under the mandate of Section 4(h) of the Regional Act, the Northwest Power Planning Council was to develop a Fish and Wildlife Program. BPA's Administrator is authorized in Section 4(h)(lO)(A) to "use the funds and the authorities available to the Administrator . . . to protect, mitigate, and enhance fish and wildlife to the extent affected by the development and operation of any hydroelectric project of the Columbia River and its tributaries". The fund is to be used to implement measures that are consistent with the Council's Fish and Wildlife Program.

It was felt that BPA's involvement in dealing with disease diagnosis and control could benefit from a focused planning effort, endorsed by the regional fishery agencies, that would better define goals and objectives within disease research. The idea for a workshop to discuss the current status of viral diseases and to define resrarch needs concerning further research activity was originally discussed between BPA and Oregon State University. From there, JoAnn Leong and Warren Groberg developed and organized the agenda, selected the participants, and handled arrangements for the workshop.

The participants to the workshop were selected on the basis of their active involvement in research, diagnosis and clinical work with viral diseases in the Columbia River Basin. The comments contained within the Question and Answer section following each presentation are the personal opinions of the invited guests, and as such do not reflect agency policies or directives.

Theresa Y. Barila

Participants

Kevin H. Amos
Washington Department of Fisheries
115 G.A. Building
Olympia, Washington 98504

Theresa Y. Barila
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97208

Wayne D. Brunson
Washington Department of Game
4912 192nd Street
Lynnwood, Washington 98036

Robert A. Busch
Clear Springs Trout Co.
P.O. Box 712
Buhl, Idaho 83316

Warren J. Groberg, Jr.
Oregon Department of Fish and Wildlife
Department of Microbiology
Oregon State University
Corvallis, Oregon 97331

Steve Leek
U.S. Fish and Wildlife Service
Lower Columbia River Fish Health Center
Box 17
Cook, Washington 98605

JoAnn C. Leong
Department of Microbiology
Oregon State University
Corvallis, Oregon 97331

Joe C. Lientz
U.S. Fish and Wildlife Service
Dworshak Fish Health Center
Box 18
Ahsahka, Idaho 83520

Dan Mulcahy
National Fisheries Research Center
Building 204
Naval Support Activity
Seattle, Washington 98115

Harold Ramsey
Idaho Department of Fish and Game
Rt. 1 Box 250
Hagerman, Idaho 83332

Leo E. Ray
Fish Breeders of Idaho
Rt. 3 box 234
Buhl, Idaho 83316

Steve Roberts
Washington Department of Game
1421 Anne Avenue
East Wenatchee, Washington 98801

John S. Rohovec
Department of Microbiology
Oregon State University
Corvallis, Oregon 97331

Gib Taylor
U.S. Fish and Wildlife Service
2625 Parkmont Lane
Olympia, Washington 98502

Einar Wold
National Marine Fisheries Service
847 NE. 19th Avenue
Portland, Oregon 97208

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The Status of Viral Fish Diseases in the Columbia River Basin [1]

W. J. GroGerg, Jr.

Oregon Department of Fish and Wildlife

Department of Microbiology

Oregon State University

Corvallis, Oregon 97331-3804

[1] Oregon Agricultural Experiment Station Technical Paper No. 6576

INTRODUCTION

There are four known viral fish pathogens which have been identified among populations of salmonid fish in the Columbia River basin (CRb). These are: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), Herpesvirus salmonis and erythrocytic necrosis virus (ENV) (Mulcahy et al., 1980). Of these, IHNV and IPNV are best known because the viruses and the diseases they cause have been extensitvly described (Pilcher and Fryer, 1980) and because they have resulted in substantial losses among trout and salmon. Herpesvirus salmonis infections in this region have been limited to a single location, the Winthrop National Fish Hatchery in the state of Washington. This virus was recovered from rainbow trout (Salmo gairdneri) brood stock in 1974, 1975 and 1976. Infected stocks were destroyed in 1976 and the virus has not been isolated since (W. G. Taylor, personal communication). Recently, ENV has been reported to be relatively widespread among salmonid stocks in Oregon (Rohovec and Amandi, 1981) and potentially a lethal pathogen for Pacific salmon (MacMillian and Mulcahy, 1979). Observations at two Oregon hatcheries suggest the virus may have been a factor in coho salmon (Oncorhynchus kisutch) losses and the impact of this agent may be more than previously realized (J. E. Sanders, personal communication).

Available data regarding isolations of IPNV from salmonids in the CRb since 1980 have been documented (Table 1). The disease was prevalent in private, commercial and state hatcheries in Idaho until recent years when its impact is reported to have subsided (Busch, 1982). It is apparently enzootic in the Pahsimeroi River and Hells Canyon summer steelhead trout (Salmo gairdneri) stocks and is occasionally isolated from trout throughout the basin.

Epizootics of IPN occurred at two Oregon hatcheries from 1973 to 1975. Spread of the disease was limited to those years and locations through stringent sanitation measures and destruction of infected stocks (Mulcahy et al., 1980). There have been only two reported isolations of IPNV from fish in the state of Washington. The first was from cutthroat trout (Salmo clarki) at Leavenworth Hatchery (USFWS) in 1962 (Parisot, Yasutake and Klontz, 1965). The second was at Tucannon Hatchery (WDG) in 1982 (Roberts, 1982). This virus was detected in a tissue pool containing kidney, spleen and pylorlc caeca from six adult summer steelhead trapped at Wells Hatchery (WDG). Coded wire tag recovery revealed that the sample contained tissue from a Snake River fish that had apparently strayed. It is probable that this fish was an IPNV carrier. This observation profoundly demonstrates how rapid dissemination of a pathogen can occur through straying of infected or carrier fish. These data indicate that IPNV is widely distributed throughout the basin and, under appropriate conditions, it may create disease problems not unlike those now being realized with IHNV.

Because there are no methods for control of any viral fish disease other than avoidance, continuous surveillance of stocks for these agents is necessary. Historical documentation of these data is important for fisheries managers and information on the occurrence and distribution of these agents is available for the state of Oregon (Mulcahy et al., 1980; Groberg, Hedrick and Fryer, 1980). Such a body of epidemiological data has not been synthesized for the CRb and it is now apparent that this would be valuable. This report attempts to start this process emphasizing recent occurrences of IHNV within the CRb.

INFECTIOUS HEMATOPOIETIC NECROSIS

Recent History

Infectious hematopoietic necrosis (IHN) can result in castrophic mortality in intensively cultured fish. The incidence of the virus in CRb hatcheries during 1980 (Table 2) is partially representative of IHNV isolations at basin facilities in preceding years. Four isolations were made at stations where the virus had previously been detected and this was not unusual. What may have been atypical, however, were two now isolations, one at Pahslmeroi Hatchery (IDFG) and the other at Dworshak Hatchery (USFWS). This was apparently the first viral examination of the Pahsimeroi adult summer steelhead and it is not known how long this stock may have been infected. In 1981 a dramatic increase in the occurrence of the virus in CRb fish was noted. This increase was apparent both as a rise in the number of locations reporting the virus from adult (carrier) fish for the first time (Table 3) and as epizootics (Table 4) at several locations, also as first occurrences of this disease. This rapid increase was cause for extreme concern, The preliminary data on IHNV in the basin thus far into 1982 (Tables 5 and 6) indicates that the virus continues to be more widely disseminated than previously. Documentation of losses to IHNV is not available from commercial and private trout hatcheries in the Hagerman Valley of Idaho. However, a recent report indicates that mortalities to IHN in 1982 have averaged 30% in juveniles and have reached as high as 70% at certain of these facilities (Busch, 1982). The widespread occurrence of the virus in the system now poses a serious threat to susceptible species reared in all CRb hatcheries. It is conceivable that the establishment of infections in many of these stocks has reached proportions such that the virus may impact certain wild populations already severely depleted.

From 1980 to 1981 losses to the virus increased by greater than ten fold (Table 7). Egg losses represent the destruction of eggs which have been compromised as a result of virus isolation from the brood fish. Fish losses are actual mortality from the disease combined with total numbers of fish destroyed. The destruction of eggs as a method of avoidance has been recommended in some cases because there is indirect evidence for transmission of virus from an infected parent to its progeny (Carlisle, Schat and Elston, 1979). Survivors of epizootics are frequently destroyed because a previous observation indicates that some proportion of surviving fish become latent, lifelong carriers releasing infectious virus only at or near sexual maturity (Amend, 1975). These losses represent severe constraints on the ability for hatcheries to meet production quotas.

Epizootiology

Epizootiology is the field of science dealing with relationships of those factors which determine the frequencies and distributions of diseases among animals (Post, 1977). Often it is not possible to unequivocally determine what specific event(s) or factor(s) have contributed to a change in the frequency or distribution of a disease. This is particularly true where epizootiological investigations involve numerous populations and races of wild or migratory animals (eg. anadromous fish) in a very large watershed. Probably the best one can hope to achieve is to develop several hypotheses and try to determine a scenario that best describes how a situation came into being. Historical documentation and new information will be required to accomplish this task. Thus, epizootiology is inherent to the study of infectious diseases in populations of organisms in order to: 1) identify the possible source of an infectious disease, 2) determine the incidence and distribution of the disease and, 3) propose possible methods for control.

Discussion has already focused upon the incidence and distribution of IHN in the CRb and possible methods for control will be the subject of forthcoming presentations. For now, then, several hypotheses will be outlined concerning the possible mechanism (source) whereby IHN has suddenly assumed catastrophic proportions in salmonids of the basin.

I. Many have proposed that IHNV was enzootic to native sockeye salmon (Oncorhynchus nerka) in the system and has therefore been prevalent for a long time. It can be argued that more intensive sampling and examination of fish for viral agents and improved detection methods account for what only appears to be an increased incidence of IHNV. Most of what this hypothesis presupposes is true and some isolations from adult fish have undoubtedly been the result of increased sampling and better detection methods. It does not account for the many recent occurrences of the virus that have resulted in epizootics in juvenile fish. It is inconceivable that losses of such proportions would have been ignored or not previously reported if the disease was widespread before 1981.

II. A second hypothesis centers around the Oregon Department of Fish and Wildlife, Round Butte Hatchery on the Deschutes River in central Oregon. In August of 1973, IHNV was isolated from spring chinook salmon (Oncorhynchus tshawtytscha) at this facility (Mulcahy et al., 1980). This was the first known occurrence of the virus at that location. Subsequently, in April of 1975, juvenile steelhead trout reared. at the same location began to die from IHN disease. All fish from tanks where the disease was confirmed were destroyed and only fish from tanks in which the disease was not confirmed were reared for release. The virus has continually been Isolated from adult chinook salmon and steelhead trout at this location in years since 1975, and with the exception of 1977 and

1981, annual losses to IHN in juvenile steelhead trout have been documented. Destruction of implicated lots has always been the policy there in hopes that virus carrier rates in returning adults might be reduced by releasing only fish from lots in which the virus was not isolated. Because there is a known IHNV infected wild population of kokanee salmon in waters immediately above the hatchery, a program of sanitation and restocking the hatchery with noninfected stocks was not undertaken. This is often referred to as the "try to live with it" approach where circumstances limit the possible avoidance measures that can be taken.

Concern was expressed within the Oregon Department of Fish and Wildlife that this infected stock could serve as a reservoir of infection for other Columbia River stocks. While this potential cannot be disregarded, it seems questionable that Round Butte Hatchery or Deschutes River stocks are the source of the virus implicated in the recent, new occurrences in the basin. This is because preliminary studies indicate that the N protein of this virus has a lower molecular weight than that of these recent isolates (J. C. Leong, personal communication) and is therefore a different strain of IHNV. Further, the virus was known to be prevalent in the Round Butte stock since 1975. It is difficult to explain why its impact would not be realized in other CRb stocks until 1981. It must be emphasized, however, that more data is needed to precisely describe strains of IHNV. These comparisons should take into account not only the molecular biology of the viruses but other properties of viral entities that provide comparative information. This should include virulence, plaque characteristics and temperature sensitivity.

III. Another hypothesis is that transfers, either knowingly or unknowingly, of infected eggs, juveniles and adults between facilities has resulted in the widespread dissemination of the virus. Along with this, potentially contaminated water and equipment have been moved frequently from one location to another and the result has been the direct introduction of infectious virus into previously uncontaminated waters. These practices have contributed to the IHN problem that now exists (Crawford, 1982) and movement of eggs, fish and equipment between facilities within the basin, as well as to and from locations outside the basin, should be discouraged. When transfers are made, they should be carefully evaluated in terms of the potential for introduction of IHNV and carried out only when absolutely necessary to enhance production. Any transfer of eggs, fish and water from the CRb to other waters must now be viewed as a high risk practice for the introduction of IHNV, even with certified (inspected) stocks.

IV. Other hypotheses have been suggested and the basis of these are the IHN infected stocks of fish at state, commercial and private hatcheries in the state of Idaho. Presumably, because destruction of infected stocks was not a policy, the virus became widespread in cultured and wild fish in the state during the 1970's. Survivors of epizootics at some state hatcheries were propagated and released. If survivors of epizootics are carriers of the virus, these fish potentially could have served as reservoirs of infection for other uninfected stocks. How this reservoir of infection impacted upon stocks of fish downriver can be developed as several hypotheses. Two will be discussed because others proposed depend on the same basic assumptions and are simply variations of these.

A. The contribution of virus to the Snake River from commercial, private and state hatcheries was such that significant levels of infectious virus were present in the water and these viruses began to infect fish in the middle and lower Columbia River. It was simply the presence of infectious virus in the water, then, that accounted for the sudden increase in IHN in downriver stocks. This proposal has some merit. However, the potential for IHNV to retain infectivity after many months in a prolonged journey downriver suspended in water seems unlikely. Further, if this were the mechanism of transmission downriver, one would expect to see a gradual progression in the incidence of the virus downriver rather than a sudden increase throughout the entire CRb which seems to have been the case.

B. A second hypothesis that can be developed focuses upon presumed IHNV carrier summer steelhead trout reared in Idaho and transported to the lower Columbia River for release below Bonneville Dam. In the late 1970's this practice was implemented in earnest to determine whether the substantial reduction of smolt mortality through dams on their downstream migration, would result in greater adult returns could be realized at hatcheries far upriver. Several million summer steelhead smolts reared at Idaho hatcheries were transported downriver for release. Since the transport program began, it has been observed that these fish, as returning adults, tend to stray to lower Columbia River tributaries at a rate much greater than that of their counterparts which migrated downstream as smolts. Reports have been made of adult traps at lower Columbia River facilities being full of upriver adult summer steelhead.

If, then, assumptions are made that 1) these adult fish strayed at a high rate, 2) there were more of them because of increased survival rates and 3) many were IHNV carriers, a hypothesis can be developed that seems to account for the recent sudden increase in IHN throughout the basin. The introduction of numerous IHNV carrier adults into tributaries where the virus was not previously established has resulted in contamination of stocks in those tributaries, either through vertical or horizontal transmission, or both. The important point that gives this proposal validity is that the increased incidence of IHNV in the lower Columbia River began in the spring of 1981 when many of the fish transported in the late 1970's would have been returning as adults. Further, drastic changes in the water chemistry in the Lower Columbia, Cowlitz and Toutle Rivers from the eruption of Mt. St. Helens is believed to have caused homing difficulties for many returning adult fish in the winter of 1980-81 (Crawford, 1982). Additionally, that upriver adults were previously infected is apparent from a review of Table 2 which shows that the only new isolations of IHNV made in that year were at Pahsimeroi (IDFG) and Dworshak Hatcheries (USFWS), both upriver in the state of Idaho. These isolations may have represented the "tip of the iceberg" for ensuing epizootics.

It cannot be over emphasized that these hypotheses depend on certain assumptions, some of which may be proven. as facts and some of which are purely conjecture. Quite probably, there are aspects of each of these or other hypotheses that could be proposed to account for recent events concerning IHN in the CRb. Much research and further investigation needs to be conducted to elucidate these

possibilities. The effort of trying to develop a reasonable hypotheses to explain the sudden rise in the incidence of IHN in the CRb is part of the epidemiological (epizootiological) investigation. Without this aspect of epidemiology, attempts for control of this viral disease in the basin might well be hopeless.

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Sanders, J. E. (personal communication). Oregon Dept. of Fish and Wildlife, Dept. of Microbiology, Oregon State University, Corvallis, OR 9733A-3804.

Taylor, W. G. (personal communication). U.S. Fish and Wildlife Service, Olympia Fish Health Center, 2625 farkmont Lane, Bldg. A, Olympia, WA 98502.

The following abbreviations are used in Tables l-6.

A. Abbreviations for management agencies responsible for facilities listed.

IDFG	Idaho Department of Fish and Game
ODFW	Oregon Department of Fish and Wildlife
USFWS	United States Fish and Wildlife Service
WDF	Washington Department of Fisheries
WDG	Washington Department of Game

B. Abbreviations for species of fish.

BT	brook trout	K .	kokanee salmon
ChF	fall chinook salmon	Rb	rainbow trout
ChS	spring chinook salmon	StS	summer steelhead trout
Ct	cutthroat	StW	winter steelhead trout

c. Abbreviations for age of fish

Juv	juvenile
Yr	yearling
Ad	adult

Table 1. Isolations of ifnectious pancreatic necrosis virus from salmonid fish in the Columbia River basin since 1980.

Facility	Major river drainage	Date	Species	Age
Warm Springs H. (USFWS)	Deschutes	2-80	sts	Yr
Pahsimeroi H. (IDFG)	Salmon	5-80	sts	Ad
Cascade Lakes	Deschutes	8-80	B T	V[a]
Oxbow H. (IDFG)	Snake	3-81	sts	Ad
Gnat Creek	Columbia	6-81	ct/st	Juv
Tucannon H. (WDG)	Snake	4-82	sts	Ad
Warm Springs H. (USFWS)	Deschutes .	5-82	sts	Ad
American Falls H. (IDFG)	Snake	ND[b]	Rb	Juv
Hagerman H. (IDFG)	Snake	ND	Rb	Juv

'Various ages from juveniles to 2+.

[b]Indicates no data available.

Table 2. Isolations of infectious hematopoietic necrosis virus from salmonid fish at Columbia River basin hatcheries during 1980.

Facility	Major river drainage	Species	Age	First known occurrence IHNV this location
Round Butte H. (ODFW)	Deschutes	sts	Ad	8-73
Warm Springs H. (USFWS)	Deschutes	sts	Ad	4-79
Round Butte H. (ODFW)	Deschutes	sts	Juv	8-73
Pahsimeroi H. (IDFG)	Salmon	sts	Ad	5-80
Speelyai H. (WDF)	Lewis	ChS	Ad	4-73
Dworshak H. (USFWS)	Clearwater	ChS	Ad	9-80

Table 3. Isolations of infectious hematopoietic necrosis virus from adult salmonid fish at Columbia River Hatcheries during 1981.

Facility	Major river drainage	Species	First known occurrence IHNV this location
Round Butte H. (ODFW)	Deschutes	sts	8-73
Cowlitz H. (WDG)	Cowlitz	stw sts ct	2-81
Warm Springs H. (USFWS)	Deschutes	sts	
Little White Salmon,(USFWS)	Columbia	ChS	8-73
Round Butte H. (ODFW)	Deschutes	ChS	8-73
Minto Pond (ODFW)	North Santiam	ChS	9-81
Speelyai H. (WDF)	Lewis	ChF	4-73
Cowlitz H. (WDG)	Cowlitz	ct sts stw	2-81
Beaver Creek H. (WDG)	Columbia	Ct	12-81

Table 4. Isolations of infectious hematopoietic necrosis virus from yearling and juvenile salmonid fish at Columbia River basin hatcheries during 1981.

Facility	Major river drainage	Species	Age	First known occurrence IHNV this location
Entiat H. (USFWS)	Columbia	ChS	Yr	6-74
Eagle H. (IDFG)	Snake	Rb K	Juv Juv	4-81
Gnat Creek H. (ODFW)	Columbia	stw	Juv	4-81
American Falls H. (IDFG)	Snake	Rb	Juv	l-80
Skamania H. (WDG)	Washougal	sts	Juv	5-81
Mossyrock H. (wDG)	Cowlitz	stw Rb ct	Juv Juv Juv	5-81
Cowlitz H. (WDG)	Cowlitz	Rb ct	Yr Juv	2-81
Niagra Springs H. (IDFG)	Snake	sts	Juv	7-78
Dworshak H. (USFWS)	Clearwater	Rb	Yr	9-80
Hagerman H. (IDFG)[a]	Snake	Rb	Juv	11-81

[a]IHNV diagnosed coincident with a proliferative kidney disease epizootic.

Table 5. Isolations of infectious hematopoietic necrosis virus from adult salmonid fish at Columbia River basin hatcheries during 1982.

Facility	Major river drainage	Species	First known occurrence IHNV this location
Pahsimeroi H. (IDFG)	Salmon	sts	5-80
Dworshak H, (USFWS)	Clearwater	sts ChS	9-80
Cowlitz H. (WDG)	Cowlitz	ct sts stw	2-81
Beaver Creek H. (WDG)	Columbia	sts stw	12-81
Skamania H. (WDG)	Washougal	sts	5-81
Kalama Trap (WDG)	Kalama	sts	3-82
Rapid River H. (IDFG)	Salmon	ChS	?-79
Leavenworth H. (USFWS)	Wenatchee	ChS	?-51a
Speelyai H. (WDF)	Lewis	ChS	4-73

a Loss attributed to an unknown filterable agent in later years identified as IHNV (Watson et al., 1954).

Table 6. Isolations of infectious hematopoietic necrosis virus from juvenile salmonid fish at Columbia River basin hatcheries during 1982.

Facility	mAJ0R river drainage	Species	First.known occurrence IHNV this location
Niagra Springs H. (IDFG)	Snake	sts	7-78
Dworshak H. (USFWS)	Clearwater	ChS	9-80
Round Butte H. (ODFW)	Deschutes	sts	8-73
Cowlitz H. (WDG)	Cowlitz	ct stw sts	
Beaver Creek H. (WDG)	Columbia	Ct stw sts	12-81
Skamania H. (WDG)	Washougal	sts	5-81

Table 7. Estimated losses of trout and salmon eggs and juvenile fish to infectious hematopoietic necrosis virus at Columbia River basin hatcheries[a] since 1980.

Year	Eggs destroyed[b] (x 1,000)	Juvenile mortality[c] (x 1,000)	Cumulative loss (x 1,000)
1980	149	150	299
	4,805	2,938	7,743
1982	1,125	5,446	6,571

a Does not include data for private trout hatcheries in Idaho which is not available for the public record.

b Eggs destroyed because IHNV recovered from brood fish.

' Loss to IHN plus fish destroyed because they were with infected fish.

Methods for Diagnosing IHNV Infection in Fish [1]

J. C. Leong
Y. L. Hsu
H. M. Engelking
J. L. Fendrick
L. K. Durrin
G. Kurath

Department of Microbiology

Oregon State University

Corvallis, Oregon 97331

1 Oregon Agricultural Experiment Station Technical Paper No. 6719

METHODS FOR DIAGNOSING IHN INFECTION

INTRODUCTION

Effective management of fish health in rearing facilities requires the use of rapid, accurate, and sensitive methods for diagnosing disease. Early diagnosis can result in the control of the disease with a decrease in mortality and a halt to the further spread of the infectious agent. In the case of infectious hematopoietic necrosis virus (IHNV), current diagnostic procedures rely primarily upon classical methods of virus isolation in tissue culture and virus identification by serum neutralization. Neither method is rapid and virus isolation in tissue culture may be inadequate for detecting the virus in host tissues. This paper reviews current methods used in viral diagnoses and discusses how some of these methods may be used for IHNV infection.

DIAGNOSIS BY DISEASE SIGNS

A typical outbreak of IHN occurs in salmon or trout fry of up to 2 months of age. The mortality rate decreases in older fish and the disease is not seen in fish of 2 years in age or older (Pilcher and Fryer, 1980). The infected fish are lethargic and exhibit some or all of the following symptoms: abdominal swelling, exopthalmia, pale gills, hemorrhages at the base of the fins and dark coloration.

Examination of the internal organs reveals unusually pale liver, spleen, and kidneys. A fluid milk is found in the stomach and the intestine is filled with a watery yellow fluid. Petechiae may be seen throughout the mesenteries and visceral adipose tissue (Pilcher and Fryer, 1980).

Presumptive diagnosis of IHN disease may be made at this time. However positive diagnosis requires Isolation of the viral agent and neutralization of the virus by specific antiserum.

VIRUS ISOLATION IN TISSUE CULTURE

The recommended method for detection of IHNV relies on the development of a characteristic cytopathic effect (CPE) on cells inoculated with tissue homogenates or fluid specimens (American Fisheries Society, 1979). The virus will grow on fish cells when incubated at 12 and 16 C and produce a CPE characterized by rounded cells in clusters with margination of chromatin at the nuclear membrane.

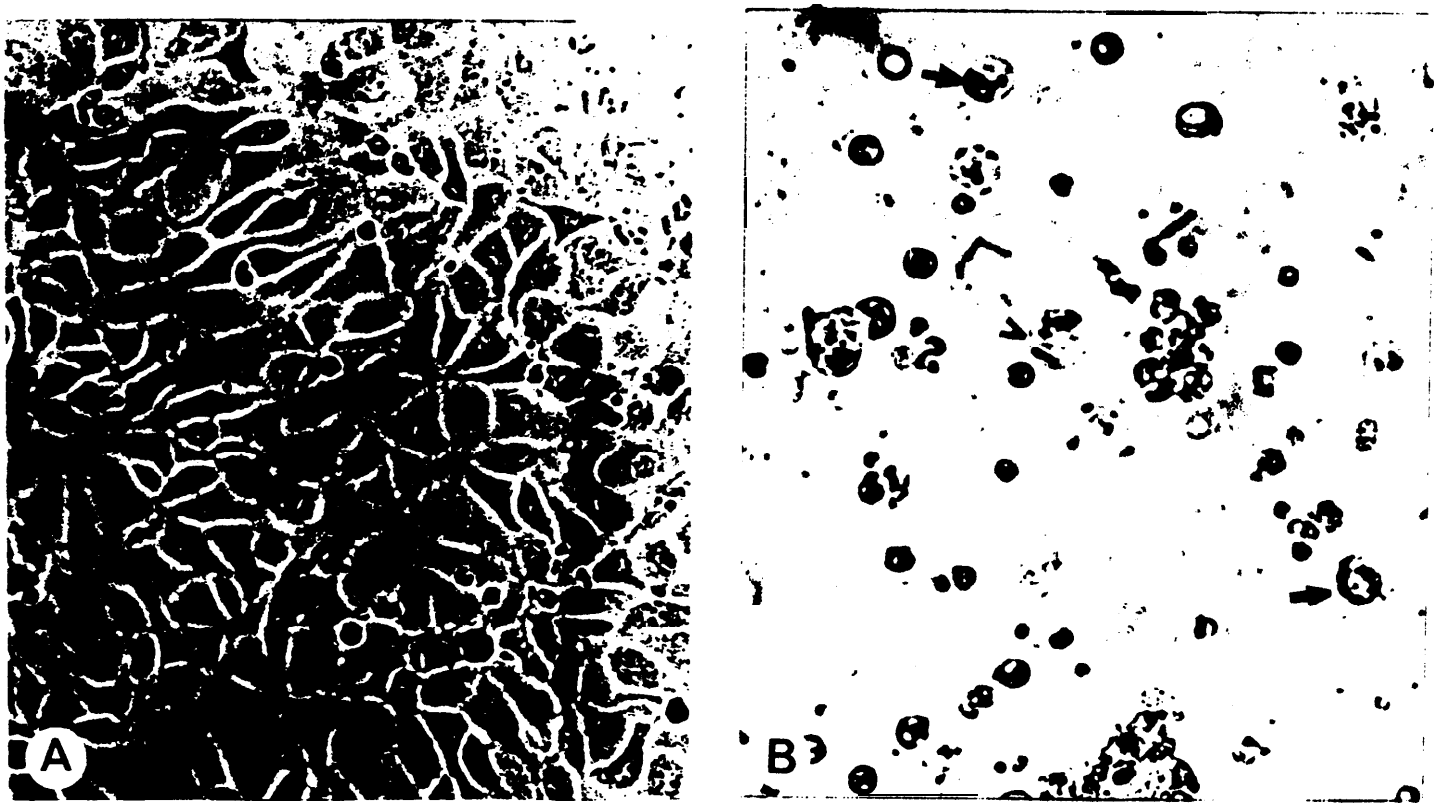


Figure 1. Characteristic cytopathic effect of IHNV in CHSE-214 (chinook salmon embryo) cells.

A. Uninfected CHSE-214 cells. B. CHSE-214 cells infected with IHNV at 72 hours postinfection. The characteristic baloon-shaped cells are indicated by the arrows.

Table 1. IHNV titers on five different cell lines.

Assay Type	RTG-2	EPC	CHSE-214	FHM	STE-137
$TCID_{50}/ml$					
Round Butte	0	1.4 x 10^2	0	9.3 x 10^1	0
Elk River	0	1.4 x 104	1.4 x 104	3.0 x 104	3.6 x 10^2
PFU/ml					
Round Butte	0	1.5 x 10^2	0	2.5 x 10^2	0
Elk River	0	1.0 x 10^6	9.0 x 10^5	1.0 x 106	NT

From Fendrick, Groberg, and Leong, 1982. RTG-2, rainbow trout gonad cells; EPC, epithelioma papillosum cyprini cells; CHSE-214, chinook salmon embryo cells; FHM, fathead minnow cells; STE-137, steelhead trout embryo cells.

The choice of a cell line for isolating IHNV is particularly important. We have shown that differences in cell line sensitivity to virus infection can lead to false-negative results. A comparison of five different cell lines for their relative sensitivity to IHNV infection from fresh samples was made. As shown in Table 1, there are remarkable differences in cell line sensitivity to IHNV infection.

In addition, the plaque assay is apparently more sensitive than the end point dilution assay for some virus isolates. For the Elk River virus, a lOO-fold higher virus titer was observed in the plaque assay. The difference in virus titer may be attributed to interfering particles which may affect the end-point dilution assay. Autointerference has been demonstrated for IHNV (McAllister and Pilcher, 1974; Engelking and Leong, 1980). A similar phenomenon has been noted for polio virus by Gabrielson and Hsiung (1965). In a comparison of both types of assay, the plaque assay was much more sensitive than the end-point dilution assay for detection for enteroviruses in clinical specimens.

In our laboratory, the CASE-214 and EPC cells are used for routine testing of samples for IHNV and IPNV. It has been our experience that these cell lines offer greatest sensitivity and reliability for detecting these viruses in fresh samples from infected fish. However, cell lines are dynamic biological entities and a particular cell line carried by different laboratories can vary widely in its growth characteristics and response to virus. All diagnostic laboratories should maintain surveillance of their cell lines' viral sensitivity and employ at least two cell lines in the viral testing.

The appearance of virus-induced CPE is monitored daily and in those samples with large quantities of virus, CPE can be observed as early as 2 days after infection (Figure 2). After 6-7 days, no new virus-positive wells appear and the $TCID_{50}$ assay is completed in one week. Samples with very low virus titers can take as long as three to four weeks for CPE induction. Thus, apparently negative cultures must be observed for at least two weeks and then blind-passaged two more times for another 4 weeks (Figure 3). Since IHN disease can spread rapidly and kill 90% of the fish population in 2 to 3 weeks the question of whether mortality is caused by IHNV becomes moot at this point. If virus-like CPE does appear in culture after this time period, standard virus isolation procedures (American Fisheries Society, Fish Health Section, 1979) require that these positive cultures be retested with anti-IHNV antisera. The confirmed diagnosis of IHNV may take as long as 7-8 weeks. Clearly, a more rapid diagnostic method for IHNV infection is desirable.

Figure 2. $TCID_{50}$ assay of IHNV on different cell lines. Semi-confluent monlayers of FHM, STE-137, CHSE-214, RTG-2 and EPC cells were prepared in 96-well plates. These cells were inoculated with IHNV-containing tissue extracts from morbid chinook salmon alevins from Elk River Hatchery. The cells were incubated at 16°C for 15 days. Each well was examined daily for viral specific CPE and scored as positive or negative for $TCID_{50}$ calculations. Since RTG-2 cells showed no COE in this study, the data are not plotted on this figure (Fendrick, J. L., Groberg, W. J., and Leong, J. C., 1982).

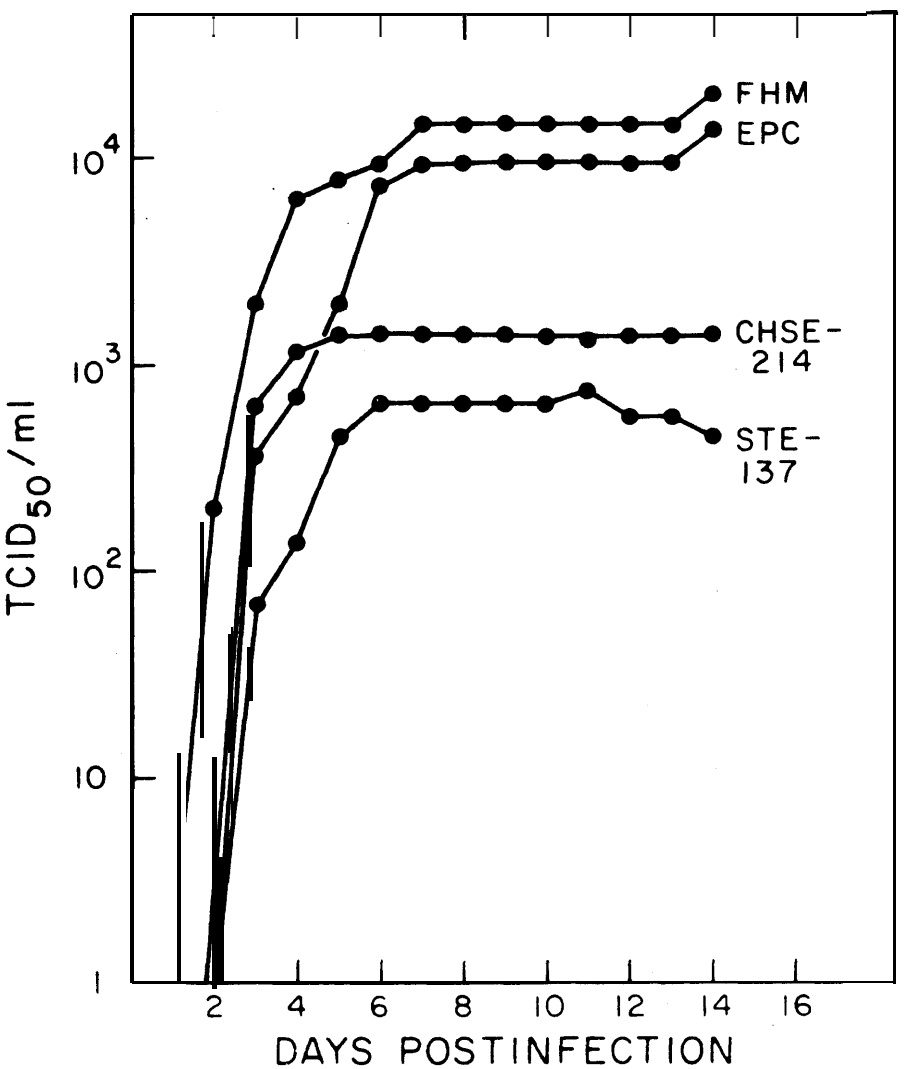
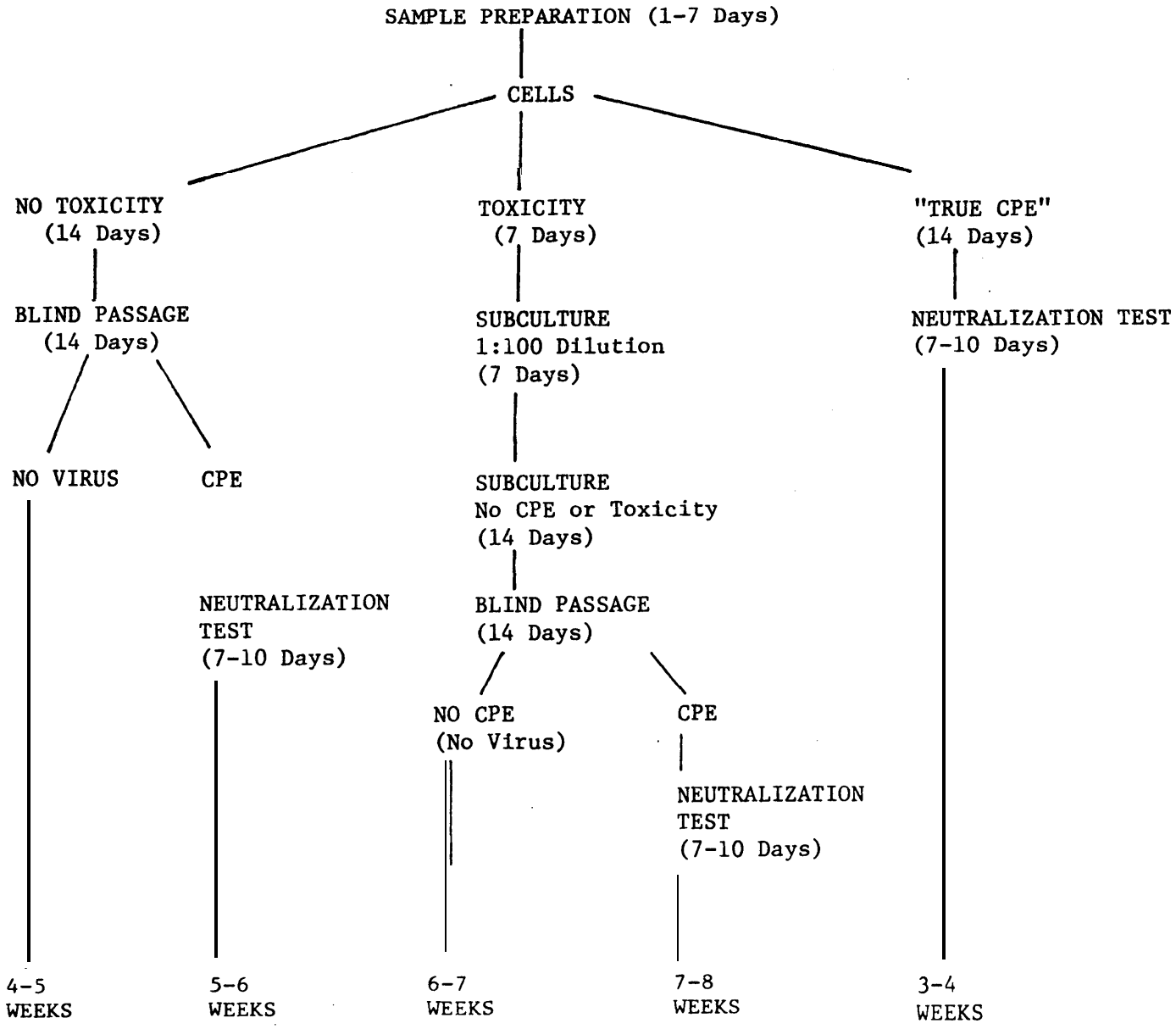


Figure 3. Time schedule for the detection of IHNV.



ELECTRON MICROSCOPY

Viruses detected by electron microscopy (EM) have characteristic shapes which make their identification feasible. Combined with the presence of pathological signs of the disease, a natural history of the disease, and a characteristic virus morphology, examination by EM can give a good initial diagnosis of a virus infection.

In recent years, electron microscopy has enabled the detection of rotaviruses in the stools of patients with viral gastroenteritis. Virions have been demonstrated in the cerebrospinal fluids of patients with herpes zoster and mumps meningoencephalitis, in the nasopharynegal secretions from patients suffering from laryngotracheitis, in the urine of infants congenitally infected with cytomegalovirus and in wart tissue (Lennette et al., 1979).

The characteristic bullet-shaped virion structure of IHNV is ideally suited for EM detection. A method was developed in our laboratory for the preliminary diagnosis of IHNV in water, ovarian, and seminal fluids by electron microscopy. It is presented here as a possible diagnostic method for further study. In this procedure, the virus-containing fluid is layered over a collodion-coated grid supported by a glass coverslip which in turn is supported by a 33% polyacrylamide gel. The sample is then subjected to ultracentrifugation at 17,000 rpm at 4 C in a Beckman SW 41 rotor for 60 min. The virus particles are deposited onto the collodion film during centrifugation, stained with phosphotungstic acid, and then examined by transmission electron microscopy. Using this technique we have been able to detect IHNV at a lower limit of 1-2 x 10^4 $TCID_{50}$ units per ml or approximately 10^6 to 10^7 physical particles per ml. The particle to infectivity ratio for IHNV is 500 to 1,500 (Durrin and Leong, unpublished data).

Figure 4. Standard procedure for rapid diagnosis of IHNV from water, ovarian or seminal fluid.

VIRUS-CONTAINING SAMPLE
(Dilution or Dialysis of
sample in STE Buffer)

Differential centrifugation of sample onto colloidon coated grids supported on 33% polyacrylamide gel at 17,000 rpm, 120 minutes, SW41 rotor, Beckman L5-65

Pipet off diluent

Lift off coverslip supporting grids with a spatula.

Grids containing sedimented virus are stained by shadowcasting or negative staining.

Examination of virus
under the Electron Microscope

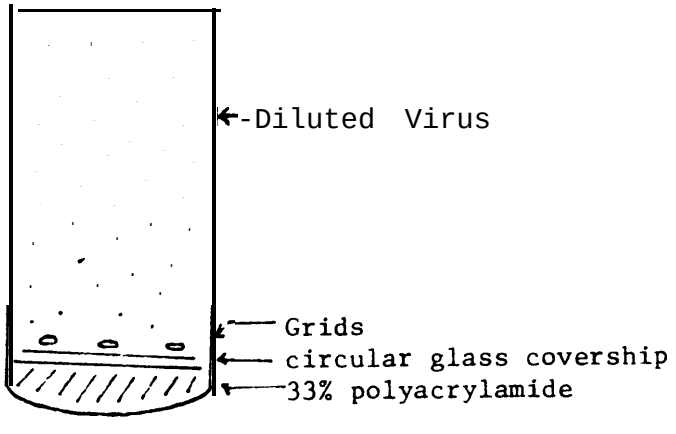


Figure 5. Electron micrographs of IHNV (A) and 0.23 micron polystyrene latex particles (B) sedimented onto collodion coated grids and developed by the shadowcast technique with platinum/paladium metal.

The diagnostic method developed here is extremely rapid. EM detection of IHNV can be accomplished within 3-4 hours after receipt of the fluid sample. The sensitivity of the technique may be acceptable if-preliminary concentration of the virus by ultracentrifugation or selective filtration is made before EM examination.

SEROLOGICAL TECHNIQUES

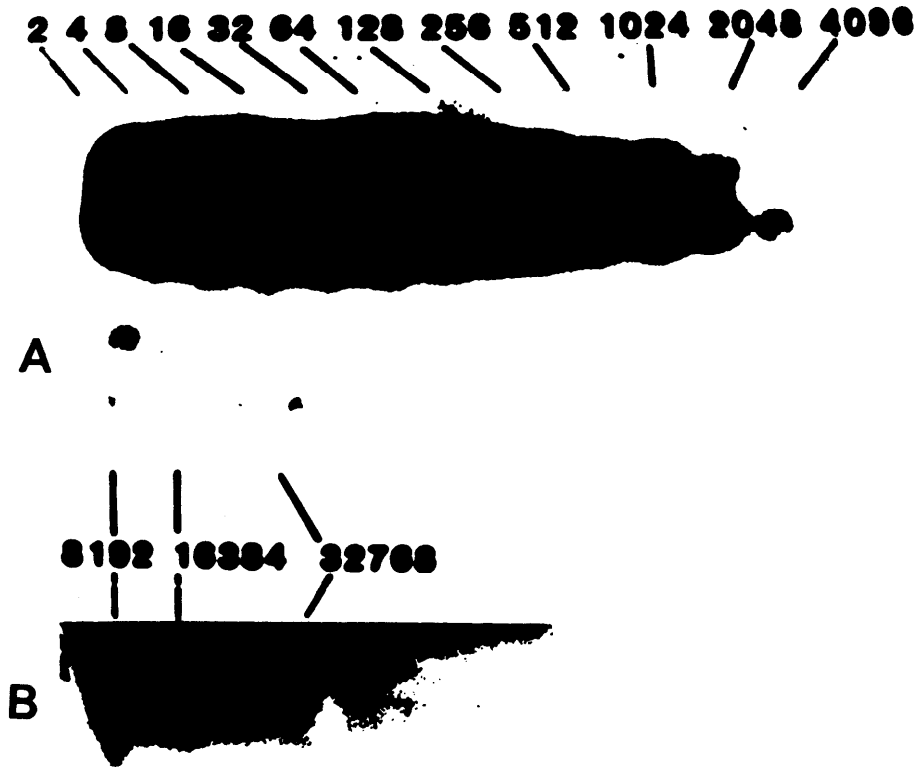
Routine serological testing for IHNV infection has been severely curtailed by the difficulties most investigators experience in obtaining antisera of suitable specificity and titer. Although serum neutralization titers of 1:3,900 (50% plaque neutralization) have been reported (McAllister et al., 1974), investigators find more typically that anti-IHNV sera from rabbits usually have neutralization titers of 1:250 or less. For this reason, immunological techniques such as radioimmunoassay, complement fixation, immunofluorescence, and enzyme linked immunoassay (ELISA) have not been used to identify IHNV. Thus, serological procedures in IHNV diagnosis have been confined to the identification of the virus by serum neutralization.

We have found that the virus neutralization titer for IHNV is not a reliable indicator for antibody titer. Antisera with a 50% plaque neutralization titer of 1:250 was found to have a titer of greater than 1:32,000 in a radioimmunoassay (Figure 6) which detected antibody-antigen. In this assay, purified IHNV (2,000 $TCID_{50}$ units per well) in 50 ul of phosphate buffered saline (PBS) was bound to the bottom of the individual wells of a 96-well Microtest II plate by incubation overnight at 37°C. The following morning the wells of the plate were blocked from further nonspecific protein adsorption by a Z-hour Incubation with 125 ul of 5% bovine serum albumin (BSA) in PBS, pH 7.2.

The antibody binding assay was performed in three steps: (1) Fifty ul of two-fold dilutions of the rabbit anti-IHNV sera in PBS was incubated in lack of the virus-adsorbed wells for 45 min at 37°C. Nonbound immunoglobulins were then removed from the wells by washing 3X with PBS containing 1% BSA. (2) One hundred thousand counts per minute of ^{125}I-labeled protein A (IPA) from Staphylococcus aureus in 40 ul of PBS was added to each of the virus-adsorbed wells for 45 min at 35°C. The residual nonbound IPA was then removed from the wells by washing with PBS. (3) The immune reactions were detected by 24-hr autoradiography of IPA-treated microtest plate in Kodak NS-ST X-ray film.

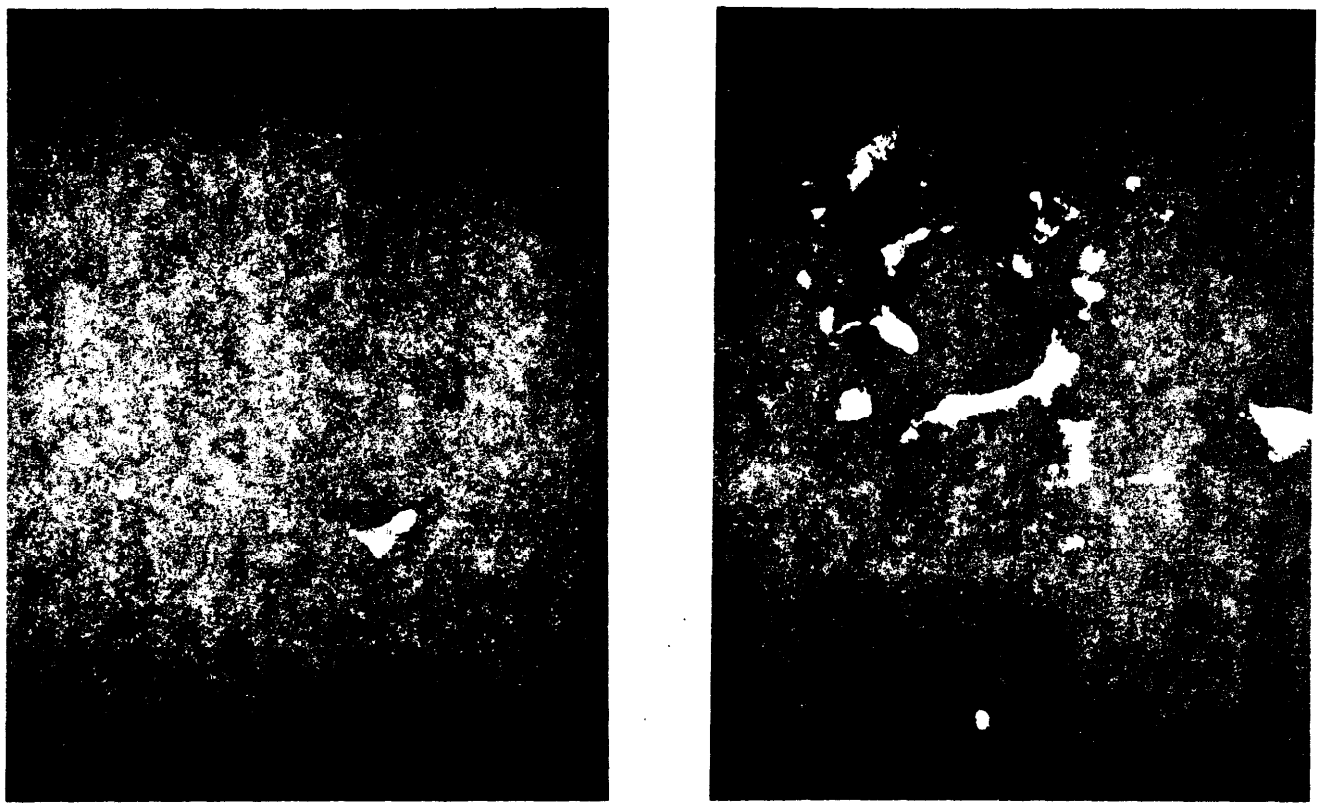
Our results show that virus neutralization may not reflect the binding titer of the antibody and suggest that rabbit anti-IHNV sera may be used for detecting IHNV by radioimmunoassay, immunofluorescence or ELISA methods. In fact, this rabbit anti-sera was used in an immunofluoresceince study to detect a single IHNV-Infected cell 12-15 hours after infection. As shown in Figure 7A, viral antigen appears to be clustered around the nuclear membrane. At 24 hours single "miniplaques" of virus infected cells are easily found in the tissue culture monolayer (Figure 78). Thus, rapid detection of viral antigens in virus-infected cells is possible within 24 hours after inoculation. The technique has yet to be tested with infected fish tissue.

Figure 6. Binding titer for anti-INN rabbit sera as determined by solid phase immunoglobulin binding with staphylococcus protein A-^{125}I. Purified IHN virus was bund to the bottom of a 96-well microtiter plate. Then varying two-fold dilutions of antisera was adsorbed to the viral antigen for 45 min at 37°C. Nonbound immunoglobulin was removed from the wells by washing with PBS. One hundred thousand cpm of ^{125}I-labeled Staphylococcus aureas protein A was added to each well for 45 min at 37°C. The nonbound protein A was removed by washing with PBS. The immune reaction was detected by autoradiography. A) 24-hour exposure B) 48-hour exposure of the same wells.



ANTIBODY TITER BY RADIOIMMUNOASSAY

Figure 7. Detection of IHNV in infected cells my specific immuno-fluorescence. Monolayers of CASE-214 cells were infected at a multiplicity of 0.01 with IHNV. A. Specific staining of a single IHN infected cell at 24 hours poetinfection. The prominent feature is the permuclear staining. B. Specific staining of a "mini-plaque" at 48 hours post infection.



A B

A likely candidate for further study is the immunoperoxidase (IP) technique to detect IHNV in infected cells. Like immunofluorescence, the IP technique offers a simple and efficient means for the detection of IHN virus and the method can be used either directly or indirectly. Horseradish peroxidase which is attached either to the viral antibody or to a gamma immunoglobulin serves as the marker. Because of the low molecular weight (about 40,000) of the enzyme, the immunoglobulin-peroxidase complex penetrates easily into the cells. A positive IP reaction can be detected by the brown product of the substrate (3.3'-diamino-benzidine). The brown pigment is a result of catalytic activity of peroxidase in the presence of hydrogen peroxide.

Although this technique has not been used with IHNV, its use for detection of IPN virus in cell cultures revealed that the direct IP technique showed less nonspecific staining and the direct method clearly gave specific results. The immunofluorescence and the IP technique were compared using the viruses IPN, SVC, and VHS as antigens in infected cell cultures. The IP technique proved to be of greater sensitivity because the antigens were detectable earlier (Faisal and Ahne, 1980).

Furthermore, the IP technique can be used for the detection of viral antigens in tissue of infected fish. The SVC virus antigen in the kidneys and spleen of infected carp has been demonstrated by means of the IP technique (Faisal and Ahne, 1980). The nonspecific positive IP reaction due to endogenous peroxidase present in the fish tissue was removed by treating the tissues with hydrochloric acid and ethanol.

The IP technique provides some benefits for diagnostic purposes.

1. Simple and quick technique
2. High specificity
3. Requires only an ordinary light microscope
4. Preparations can be kept as permanent records.

OTHER DIAGNOSTIC METHODS

We have found that different strains of IHNV can be distinguished by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the virion polypeptides (Leong et al., 1980). Major strain differences are found in the molecular weights of the envelope glycoprotein, G, and the nucleocapsid protein, N. The method that was developed to detect these differences involved labeling the intracellular viral polypeptides with ^{35}S-methionine. After a one hour labeling period, the cells were disrupted with a urea-NP40 buffer and the lysate was applied directly to the gel. The method required very little virus, one 35 mm petri dish monolayer of cells, and 100 microcuries of ^{35}S-methionine. The results were obtained within 24-48 hours after virus infection.

This technique has enabled us to begin an epidemiological study of IHNV, an undertaking which had previously been impossible. We could now ask whether virus strains are typical of certain geographical regions or species of fish and whether the introduction of a new virus strain into a region could be determined by IHNV strain typing. Although the results are preliminary, it seems that a particular watershed will have only one type of virus. That same virus strain will be found in several different species of fish in that region. For example, we compared the Warm Springs, Round Butte, and Suttle Lake isolates from the Deschutes River watershed in Central Oregon with the Nan Scott Lake and Elk River isolates in *Oregon* (Figures 8 and'9). The Deschutes isolates are similar even though the Round Butte and Warm Springs

Figure 8. Location of the sites in Oregon, Washington, and California where IHNV has been isolated.

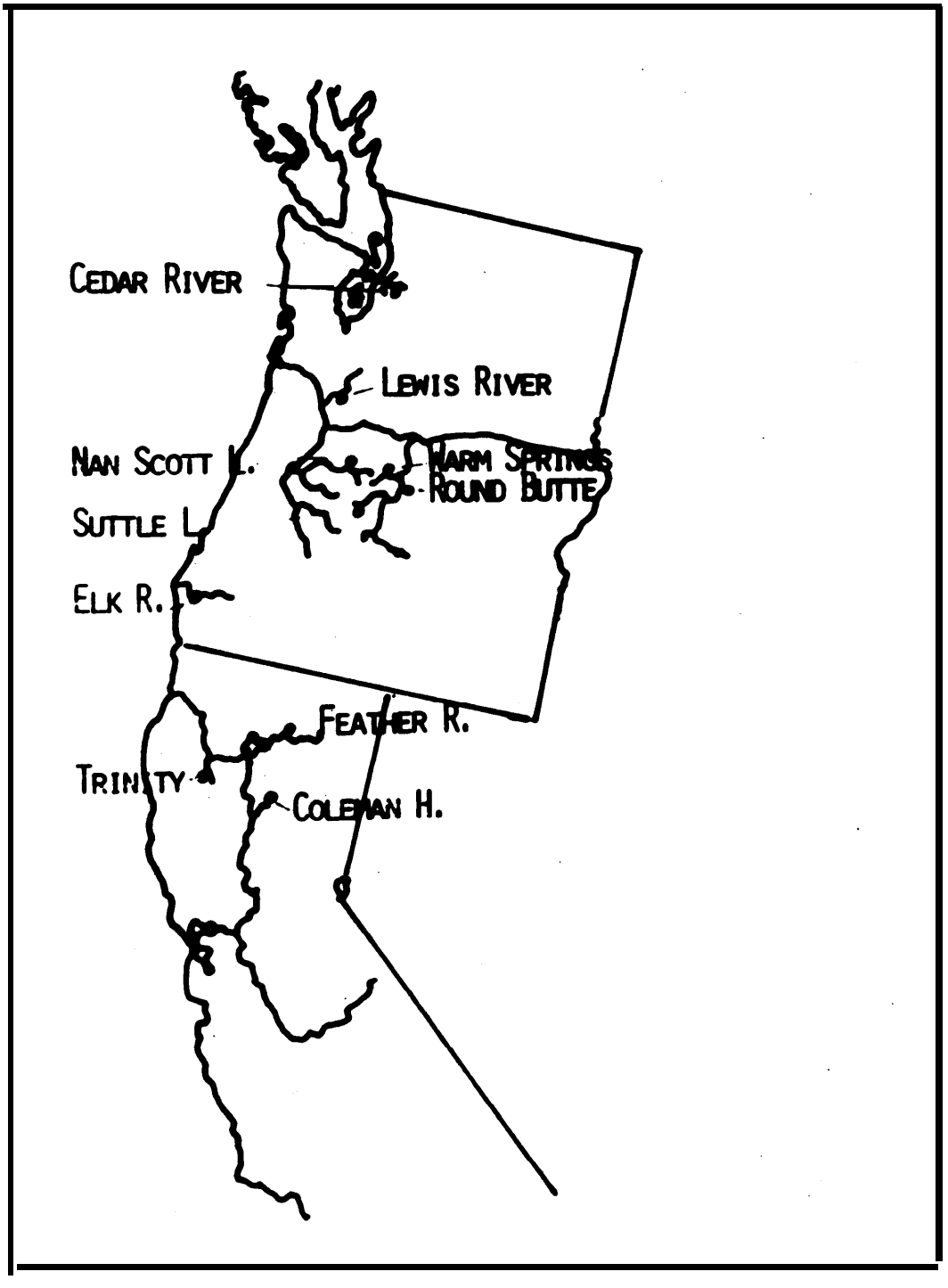
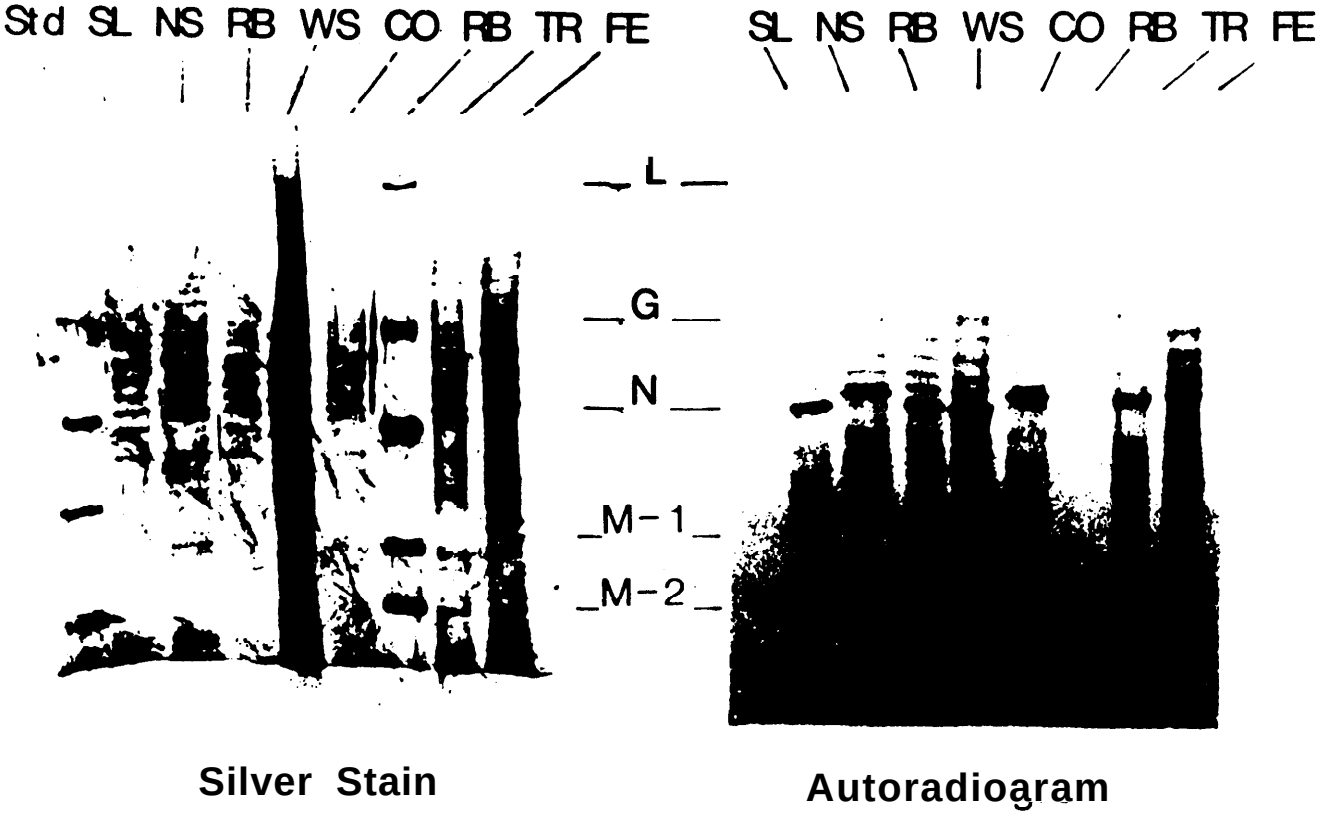


Figure 9. A comparison of eight different strains of IHNV was made by silver stain and autoradiography in SDS-PAGE. Monolayer cultures of CHSE-214 cells in 35 mm petri dishes were infected with different strains of IHNV. After 22-24 hours,, the cells were washed with methionine free MEM and labeled with 100 uCi/ml of ^{35}S-methionine for 1 hour. The samples were then processed as described. The gel on the right was developed with a silver stain. The gel on the left is an autoradiogram of the same gel. The lanes are marked from left to right Std (standard protein markers, only stained with silver), SL (Suttle Lake), NS (Nan Scott Lake), RB (Round Butte), WS (Warm Springs), Co (Coleman Hatchery), RB (Round Butte, purified virus, non-radioactively labeled), TR (Trinity River), and FE (Feather River).



isolates were taken from steelhead trout fry and the Suttle Lake virus was isolated from the ovarian fluid of spawning kokanee. Infected kokanee in the Metolius River which drains Suttle L&e have the same virus strain as well (data not shown). In addition, Round Butte isolates from steelhead fry in 1975, 1981, and 1982 show identical patterns (data not shown). These results indicate that a virus strain is endemic to a region and can remain there for years as a persistent threat to hatcheries in the region.

It is interesting to note that the Suttle Lake isolate was taken from wild stocks of kokanee upstream from the Round Butte hatchery' in late September and outbreaks of the same strain of IHNV appear in the fry at Round Butte in March of the following year. It is tempting to conclude that the wild stocks of kokanee in the Metolius River serve as a reservoir of infection for Round Butte Hatchery. However, kokanee fry have not been found with IHN disease and the Round Butte Hatchery water is obtained as seepage from springs which came into existence when the dam above the hatchery created a new lake. Thus, a simple explanation is not possible.

More recently, we have begun to type the virus samples isolated from IHN epizootics along the Columbia River watershed (Figure 11). All these isolates appear to be similar and differ from the Deschutes River strains from Warm Springs and Round Butte (Figure 12). In all, 21 different isolates of IHNV have been typed and each isolate has been grouped together with similar viruses as shown in Table II. There is no apparent species-specifiti virus strain. Instead, there are virus strains that appear to be characteristic for a geographical area. These studies suggest this technique may be a powerful tool for typing strains for a given area. Once these characteristics are recorded they can be routinely checked to monitor the introduction of new virus strains into an area.

Table II. STRAIN TYPING OF IHNV BY VIRION PROTEIN PATTERNS

AREA	STRAIN GROUP	SAMPLE DESCRIPTION	
Washington	Cedar River	Sockeye	Adults
"Columbia River"	Lewis River	Chinook	Adults
	Gnat Creek	Steelhead,W,S	Juveniles
	Beaver Creek	Steelhead,W,S	Adults
	Little White Salmon	Chinook	Adults
	Minto Pond	Chinook	Adults
	Pahsimeroi	Steelhead, S	Adults
Oregon - 1	Suttle Lake	Kokanee	Adults
	Warm Springs	Steelhead, S	Adults
	Round Butte -1975	Steelhead, S	Juveniles
	Round Butte -1981	Steelhead, S	Juveniles
	Round Butte -1982	Steelhead, S	Juveniles
Oregon - 2	Elk River	Chinook	Juveniles
	Nan Scott Lake	Rainbow	Adults
California - 1	Trinity	Chinook	Adults
	Feather	Chinook	Juveniles
California - 2	Coleman	Chinook	Adults

Figure 10. Location of the sites along the Columbia River watershed where IHNV have been isolated and characterized by protein determination.

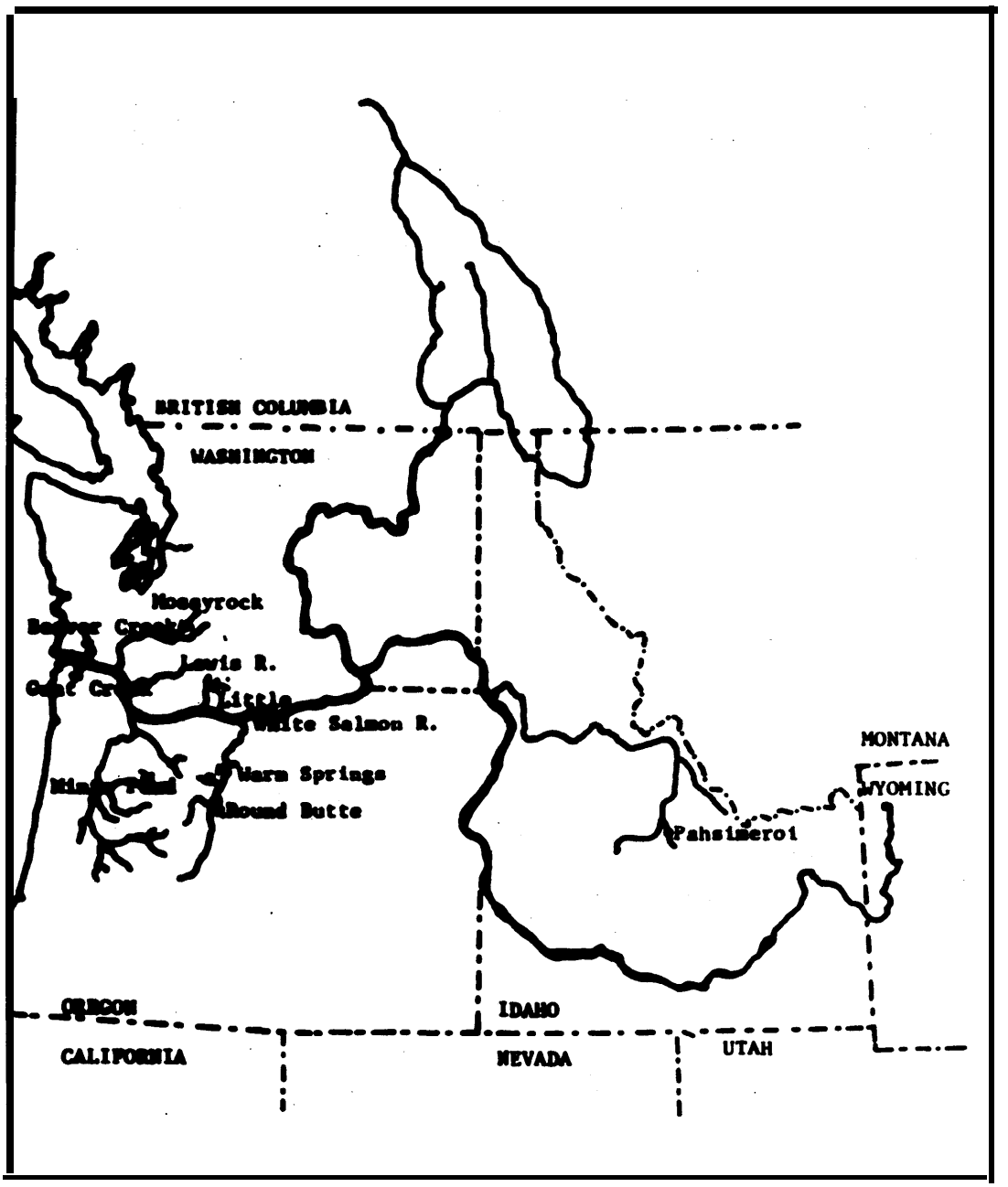
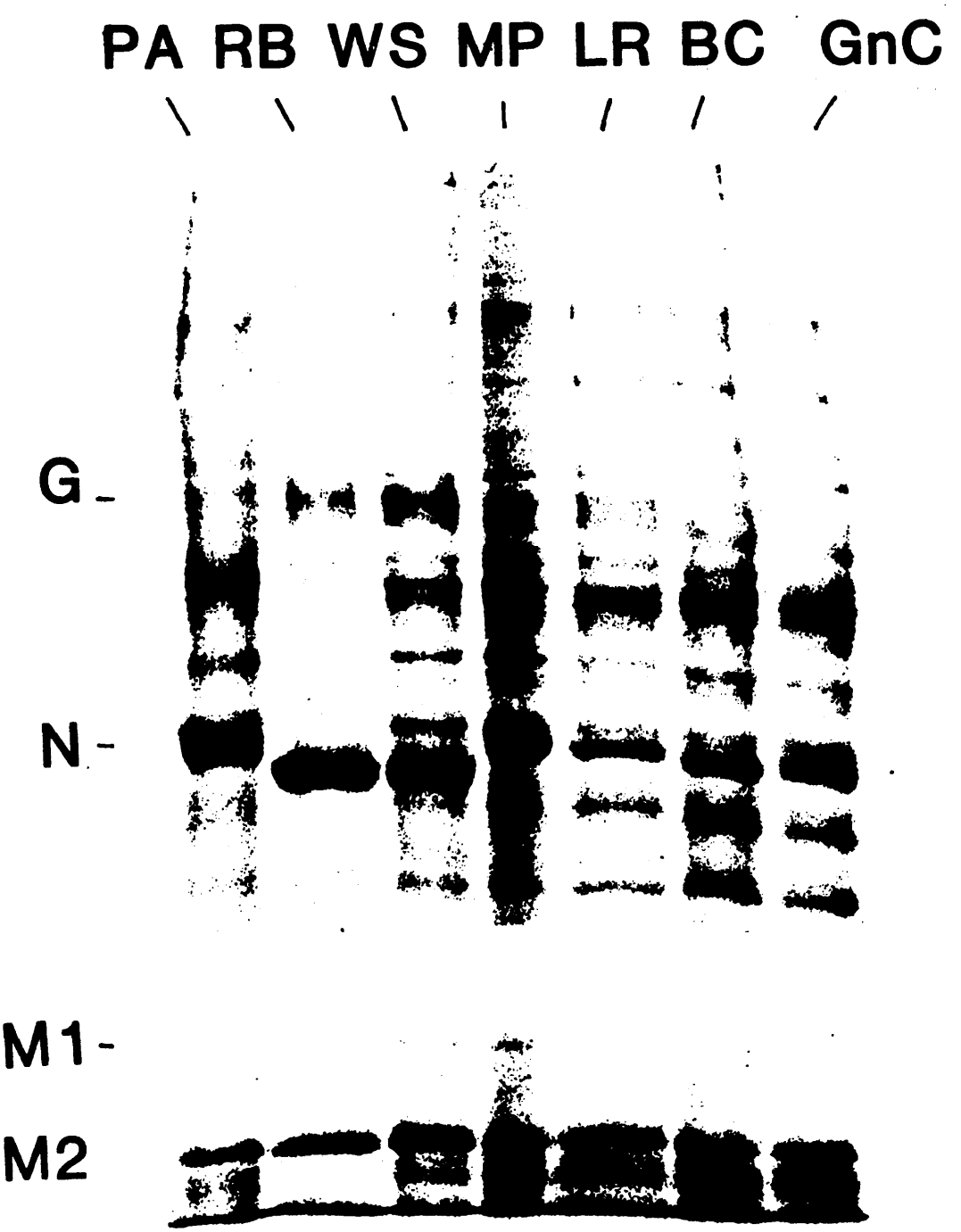


Figure 11. A comparison of seven different strains of IHNV was made by autoradiographic analysis of the intracellular proteins labeled by ^{35}S-methionine. Infected cells were labeled as described in Figure 9. The lakes are marked from left to right as PA (Pahsimeroi), RB (Round Butte), WS (Warm Springs), MP (Nubti Pond), LR (Little White Salmon River), BC (Beaver Creek), and GnC (Gnat Creek).



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Questions and Answers Following J. Leong's Presentation

D. Mulcahy We have found that there is considerable variation from individual to individual rabbit in antisera titer to IHNV. The titers we have measured range from 1:256 to 1:4,096 in a fifty percent plaque neutralization assay. It is also important that the antisera be monitored routinely and early after a booster shot because the titer plummets rapidly.

J. Leong Similar results have been reported by Hill in 1981. (Hill; B.J., Williams, R.F., and Findlay, J. Preparation of antisera against fish virus diseases agents. Develop. Biol. Standard 49:209-218, 1981)

D. Mulcahy In immunoperoxidase tests, what concentration of virus is required for identification of the virus? Would you have enough in the ovarian fluid?

J. Leong Approximately 10^1 to 10^2 virus particles/ml of fluid can be detected by this technique. Of course it is ten-fold less sensitive than virus neutralization but it is so much faster to run these tests. Normally, the inununoperoxidase test cannot be used on direct examination of the sample. However, you can get rid of the peroxidase in tissues by treatment with hydrochloric acid. This treatment maintains antigenicity and kills tissue peroxidase. The tissue can then be used directly. The direct examination of ovarian fluid may still require virus ieolation in cell culture.

E. Wold Can you use the electron microscope method for surveying water supplies?

J. Leong That's the reason it was developed. However, the procedure has never been tested under real hatchery conditions.

E. Wold Do you have an estimate of the cost for surveying a hatchery using this technique?

J. Leong It is relatively cheap. You must have the equipment on hand and electron microscope time is $35/hour.

E. Wold Has anyone approached you for doing a survey?

J. Leong No.

E. Wold What is the limit of detection by this procedure?

J. Leong We have been able to detect 10^2 to 10^3 virus particles per ml of water. However, if we combine this procedure with methods we have developed to concentrate virus from water, we can detect approximately 10^{-1} to 10^{-2} virus particles per liter of water.

E. Wold That's presumptive for a particular virus particle shape? It doesn't identify the particle.

J. Leong Yes.

D. Mulcahy We have concentrated our efforts on developing an ELISA test for IHNV because we can use it with primary samples, rather than on cell culture. Particularly when looking at 10,000 samples, ELISA seems most suitable for large scale tests.

J. Leong Have you tried it?. Have you been able to get good results?

D. Mulcahy We have for IPN virus. We dropped it because of the antiserum problem with IHN virus.

J. Rohovec Have you tried with the antiserum you have?

D. Mulcahy No. We're doing fluorescent antibody staining with this antiserum and its working quite nicely. We've found two things with our FAB studies:

1. In the indirect test, for a double antibody, instead of anti-antibody for the second antibody, we've substituted fluorescein conjugated staphylococcus aureus protein A. This technique has cut down on background staining.

2. For a counterstain, we use Eriochrome black, a metallic stain. It requires just a dip procedure and the results are nice. You can see specific fluoresence.

J. Rohovec We didn't have much luck with ELISA. There was too much non-specific staining. It worked, but sometimes negative samples would be positive.

D. Mulcahy You're talking about direct examination of the samples?

J. Rohovec I think we were using tissue culture-infected cells,.

J. Leong Dan, what is the price of the fluorresecein-conjugated Staph protein A?

D. Mulcahy Less than $100 per ml and it goes a long way.

J. Rohovec We use Evan's Blue for bacterial kidney disease (BKD) counterstaining. It's worked well and is cheap.

D. Mulcahy The definititive state of the art for fluorescent antibody staining is IPN fluorescent antibody staining. IHN FA staining looks terrible in comparison.

What I fear about using the immunoperoxidase, FA, and ELISA, tests is the technological blockade in our diagnostic labs. FAB for IHN was described before 1970. Who's used fluorescent antibody stains against IPN for examining a diagnostic sample on cell culture on primary isolation? None of us.

W. Groberg Diagnosis with IPN is no problem because neutralization results are obtained in 2 to 3 days.

D. Mulcahy Yet, the FAB tests can be done in 2 hours. However, for most of us it takes too much time and trouble to use FAB.

Control of Mortality Caused by Infectious Hematopoietic Necrosis Virus

Dan Mulcahy

U.S. Fish and Wildlife Service
National Fishery Research Center
Building 204, Naval Station Seattle
Seattle, Washington 98115

Principles for the control of IHN mortality.

Mortality caused by infectious hematopoietic necrosis (IHN) virus can be prevented by following two basic principles: never use eggs taken from infected broodstock and never raise fish in a water supply contaminated with IHN virus. A corollary to these two principles is that virus remaining in the culture facility after a viral epizootic must be removed before the two principles can be successfully applied. Presently, these principles offer the only assured method for controlling IHN mortality. They reflect the only modes of transmission known to occur for IHN virus: not using eggs from infected broodstock eliminates generation-to-generation (vertical) transmission, and using virus-free water prevents individual-to-individual (horizontal) transmission. The effect of the corollary is also to prevent waterborne virus transmission.

Extermination and quarantine as methods for control of viral diseases.

Other viral diseases of humans and other animals have been controlled by use of methods requiring a combination of direct and indirect intervention. Not all of these have yet found application to fish viral disease control. The oldest known method is the quarantine of infected populations. In animals this is often coupled with extermination of the infected stock animals. The purpose of both these procedures is to limit the spread of infection and to maintain most of the host populations free of the disease. Both quarantine and extermination have been used to control the further distribution of the fish viruses. Undoubtedly, the extermination of virus-infected fish populations will remain the most important control of fish virus diseases in geographic areas traditionally free of these diseases, and for occasions when the viruses are found in new host species.

There are difficulties with these procedure6 which severely limit their desirability. Quarantine doe6 not eliminate the mortality caused by the disease in the affected population, and there remains a chance for the spread of the contagion outside the affected population. Extermination of infected stocks is a drastic solution, since it is, in effect, killing the patient to cure the disease. However, disease control in salmonid fishes fall6 into the classification of herd medicine, in which the individual animal is secondary to the population.

In essence, extermination should be used wherever there is a chance of eliminating the disease from the host population or watershed. If successful, extermination can obviate all future costs of viral diseaese control. Fishery agency personnel often find extermination to be distasteful. Extermination causes a temporary decrease in the population and is the antithesis of a hatchery's primary function. Also, there is a tendency to view hatchery production on an annual basis, much like the dividends of a publically-owned company. Extermination must be viewed a6 being a long term investment, with the concern in one year being an investment which will allow production to proceed in future years. Unfortunately, fish killed are more easily counted than fish saved, and fish pathologists must be prepared to convince an often reluctant audience of manager6 of the wisdom of this approach.

Vaccine6 for the control of viral diseases.

Vaccines are perhaps the most widely known means of controlling viral diseases. While invaluable, in some instances vaccines have received perhaps more credit than they deserve. Public health measures such as increased sewage treatment and development of pure drinking water supplies were invaluable in reducing the incidence of such scourges as polio before the introduction of vaccines. Vaccines have become invaluable, of course, particularly for the protection of individual animals and humans.

There are two kinds of viral vaccines: attenuated ("live") and inactivated ("killed"). Attenuated vaccines consist of a strain of virus which has been weakened to the point that although still infectious, it no longer causes severe disease. The inactivated vaccines consist of virulent virus which has been killed chemically (often with formalin). Although there are many examples of successful vaccines of both types, the attenuated vaccines are considered to be the more desirable type. The attenuated vaccine strain replicates in the vaccinated host, increasing the antigenic volume at no cost to the vaccine producer. However, a full dose of inactivated virus must be delivered to the host when the killed vaccine is used. There are also differences in the immune response to each type of vaccine.

Implications for the use of vaccines for control of IHN.

Although there is considerable interest in the development of a vaccine against IHN virus, there are additional concerns as to the type of vaccine which will be used. These concerns arise from the potential impact of an attenuated IHN virus vaccine on the present control methods. Virtually all of methods presently used to combat IHN virus (broodstock culling, selection of virus-free populations for use as broodstock) or to prevent its introduction (certification and surveillance of fish stocks) rely on the isolation of IHN virus from fish. If an attenuated, live IHN virus vaccine 'is used anywhere in a watershed it immediately renders all procedures involving virus isolation moot, since it will be 'difficult, if not impossible, to determine whether an isolated virus is the vaccine strain or the wild type, virulent virus without elaborate tests. The use of an attenuated vaccine should be considered only after all populations within a watershed are determined to be infected with IHN virus, and then only when all other conceivable control methods have failed. In view of the fact that the dominant philosophy of IHN virus control

is to prevent its introduction into fish populations and to eliminate its presence, the use of attenuated vaccines at least for the near future, should be anathema to fish health professionals.

Most of the objections to an attenuated IHN virus vaccine do not hold for an inactivated vaccine. If an efficacious inactivated vaccine can be developed, even the costs could be reduced. For example, it may not be necessary to use the entire virus particle in the vaccine. It should be possible to vaccinate with the virion proteins responsible for the host immune response. If that is the case, then recombinant DNA techniques such as gene splicing may allow the inexpensive production of the desired protein as a byproduct of a bacterial fermentation.

Whatever type of vaccine is developed, a problem in its use is that it must be used prophylactically, that is, in anticipation of the viral epizootic. That means that the investment in the vaccine must be made before the need for it is proven. While in some populations there may indeed be a predictable annual epizootic, in other populations it is not unknown for the virus to be present in some years without causing a severe epizootic. Also, vaccination may not be possible for those populations which die very early in development. There is a limit on the earliest age at which fish can be vaccinated with success. Also, it takes time for immunity to develop, and an epizootic may occur before sufficient immunity has developed. The later is especially true of sockeye salmon (Oncorhynchus nerka) that die as they are emerging from the gravel, and for some groups of steelhead and rainbow trout (Salmo gairdneri) in which epizootics have occurred in alevins.

Control of IHN mortality by changing the host species being cultured.

As a disease of salmonid fishes, IHN was recognized in the 1950's when hatcheries were built in the mid-Columbia River to mitigate the loss of

spawning grounds after the construction of Grand Coulee dam. These hatcheries attempted to raise sockeye salmon for several years but several problems were encountered, not the least of which was large-scale mortality due to IHN virus. The result was that those hatcheries quit raising sockeye salmon and switched to other species. Avoiding the IHN mortality problem by eliminating the target species has been a common coping mechanism, and other examples of hatcheries which were built for one species but which changed to other species after severe problems with IHN can be given.

Control of IHN mortality by the use of elevated water temperature.

About the same time the mid-Columbia hatcheries were experiencing IHN problems with sockeye salmon, Coleman National Fish Hatchery in the Sacramento River drainage of California was losing substantial numbers of chinook salmon (0. tschawytscha) fingerlings to IHN (then known in that area as Sacramento River Chinook Disease), Although some mortality due to that disease still occurs on an annual basis at Coleman Hatchery, massive mortality is unusual because an effective treatment for the disease was discovered. Mortality can be prevented or even stopped by raising the water temperature to 14 C. This method has not proved to be effective in hatcheries located anywhere except in the Sacramento River drainage. Recent tests comparing the growth of isolates of IHN virus over a range of temperatures have demonstrated that the Sacramento River strain of IHN virus is the only one that is markedly temperature-sensitive (Figure 1). All of the strains tested showed decreased growth as the temperature increased, but only for the Sacramento River. strain does the decrease in growth occur at a sufficiently low temperature to be practical for use in fish culture. Another disadvantage of this method of control is that heating hatchery water supplies is costly unless geothermal or inexpensive heated water is available.

Control by limiting the distribution of the disease.

Traditionally, the most useful procedure for coping with IHN virus has been to limit its distribution. This has been done by instituting a scheduled program of stock inspection and surveillance. If IHN virus is found in a new location or population, the reaction varies, depending on the philosophy of the government agency or private organization involved. Reactions range from doing nothing to immediate destruction of the contaminated stock, followed by disinfection of the facility. That surveillance has been effective as a procedure is evidenced by the fact that IHN virus is not found in all populations in such drainage basins as the Columbia River. Of greater concern for the future is the lack of agreement on the proper course of action when IHN virus is found. Most fish pathologists would agree that the presence and release of increasing numbers of carrier fish in the Columbia River drainage system is undesirable and constitutes a threat to all salmonid aquaculture in the system.

The need for a drainage fish disease policy,

Although it might seem a difficult task, an effort should be made to obtain agreement on a fish disease policy among the agencies and organizations concerned with fish in the Columbia River. This does not mean that there should be any mandated action, especially for populations already affected by IHN virus. The goal should be to limit the expansion of the disease distribution as much as possible. The elimination or at least control of the disease in presently affected populations should be pursued as a separate, future goal, when effective control procedures are developed.

A problem in obtaining agreement on a course of action to limit the spread of the disease is that there is not unanimity even among fish pathologist6 as to what is proper, needed, or effective. There is

insufficient information available on the mode6 and relative importance of transmission of the virus. Too many unanswered questions exist such as: are we certain that survivors of an epizootic become lifelong carriers? If they do, what proportion of the population becomes carriers? Other than infected salmonids, is there a reservoir for the virus in freshwater? What is the efficiency of vertical transmission and how does it occur? What is the significance of the presence of virus carriers in a hatchery water supply? How does waterborne virus infect fish and what level is significant in the water? What is the relative importance of vertical and horizontal transmission? Do released or escaped carrier fish spread the virus to other free-living fish?

Many more unanswered questions can be listed, all of which are significant in determining the proper course of action. It is certain that the answers to all of these questions will not be available to decision-makers attempting to arrive at a mutually acceptable policy. A6 for most things in life, decisions will have to be made with the available basic information. Flexibility will be important to allow for changes as new information comes to light.

Broodstock culling as an experimental control procedure.

In response to the urgent need to reduce mortality of Columbia River hatchery fish, several attempts were made in the 1981-1982 spawning season to avoid using IHN virus carrier female fish as broodstock. The technique, now referred to as broodstock culling, consisted of stripping eggs from‘single female fish or from pools of three to five females, taking a sample of ovarian fluid, and maintaining the eggs in isolated incubators until the results of virus testing are available. Then, the eggs from females identified as virus carriers are removed and destroyed. The remaining eggs are then pooled and normal hatchery procedure6 followed. 58

Fish at the Washington Department of Game's Cowlitz Steelhead Hatchery had experienced severe mortality due to IHN in the 1980 brood year. In the 1981 brood year, a broodstock culling experiment was begun. All testing was done on individual females, and all samples were examined without initial dilution using the plaquing method. Fish at two other hatcheries were also culled, but as groups of several females, and using the virus isolation method, usually with a preliminary dilution of the sample. Because these are production hatcheries, and because the presence of IHN virus anywhere in the hatchery represented a threat to all of the fish at that location, no unculled controls were included in the experiment.

About 1500 female steelhead trout (summer- and winter-run stocks) and anadromous cutthroat trout were screened during the experiment. Because the infection rate was unknown, the original plan was to combine all of the eggs from carrier fish and non-carrier fish in two separate lots, anticipating the possibility of high mortality in the infected eggs. However, the infection rate was found to be low enough to permit destruction of the infected eggs,

The determination of the IHN infection rates in the Cowlitz fish was perhaps the first time in recent history that such infection rates were determined on the basis of individual fish sampling of a major Columbia River population. In anadromous cutthroat trout, the overall infection rate was 15%, in summer steelhead it was 18% and in wfnter steelhead it was 21% The incidence of virus varied from week to week in all three populations (Figure 2). The summer steelhead showed a steadily increasing infection rate throughout the spawning season, while cutthroat trout and winter steelhead had large fluctuations over their spawning seasons.

The distributions of virus titers determined for the individual fish tested showed some variations between the three populations studied (Figure

3). Most of the cutthroat trout had generally lower levels of virus than the two steelhead groups, with only 11% of the cutthroat trout having levels of virus greater than 10^5 plaque-forming-units (pfu) per ml. We consider 10^5 pfu/ml to represent an arbitrary division point between "low" and "high" titers because it is about the midpoint of the titers typically found in fish tested in the past. The mean viral titer in the cutthroat trout was 1.7×10^2 pfu/ml. The distributions of titers in both steelhead groups were similar to each other, with the proportion of titers exceeding 10^7 pfu/ml being 17% and 18% for the summer and winter steelhead, respectively. The mean titers for the steelhead groups, 1.22×10^3 pfu/ml for the summers, and 1.7×10^3 pfu/ml for the winters, were about ten-fold higher than the mean for the cutthroat trout.

Some of the female fish spawned at Cowlitz Hatchery during the broodstock culling experiment were classified into broodyear classes based on the number of years spent in the ocean, as judged by body length. Comparison to scale readings indicated this method to be correct 90-95% of the time. Variations in IHN infection rates were found between fish of different brood years returning to spawn in 1981-82 (Table 1). Not all spawners were classified by the number of years spent in the ocean, so that the number of fish classified was less than the total number of virus-positive fish in the entire experiment. Nevertheless, this subsample appeared to be representative, as judged by the close correspondence between the overall infection rates (Figure 3) and the infection rates in the subsample (Table 1). It must be emphasized that these infection rates are only statistical estimates of the true population incidence, and are likely to show variation which might be considerable.

Determlning the annual infection rates according to ocean-years subpopulations is useful for two reasons: first, in being able to roughly

predict future infection rates, one can estimate the number of fish required to obtain the desired number of eggs; second, valuable information is gained to help resolve the question of whether there is waterborne (horizontal) spread of the virus between returning adult salmon. Some concern has been expressed that the increase in infection rates over the spawning season seen in some populations might be due to infection of returning, uninfected adults by virus released from an unknown reservoir or from a small population of the returning adults who are true life-long virus carriers. If these concerns are valid, the infection rates of fish from different brood years should increase simultaneously, as they have received an identical exposure. The overall infection rates for each subpopulation should also be the same if they are exposed to the same extrinsic source of infection. However, the differences seen in the overall infection rates, and the variations in weekly infection rates over the spawning season between the fish originating in different brood years suggest that the infections are due to a lifelong carrier state, not horizontal infection (Figure 4).

Although it was not possible to include an unculled control group of fish at Cowlitz Hatchery, the other steelhead hatcheies on the Columbia River served as controls. Some culling was done at several of these hatcheries, but those efforts used a different methodology. Following broodstock culling at Cowlitz Hatchery, mortality of fry and fingerlings due to IHN was about 4% in cutthroat trout, 8% in the summer steelhead, and 14% in winter steelhead. Mortality rates at other steelhead hatcheries were typically in the range of 60 to 97%.

It is important for future work to speculate on the reasons for the differences in mortality rates, especially between that experienced at Cowlitz Hatchery and those at the other hatcheries where broodstock culling was

attempted. One variable may be the use of surface water which might be contaminated with IHN virus released by feral fish spawning above the hatcheries. Another possibility is that there were differences in the assay methods for detecting carrier fish. We believe that by screening individual spawners, using undiluted samples and the plaquing method, the likelihood of missing carrier fish with low virus titers was reduced at the Cowlitz Hatchery.

Clarification of the possible role of waterborne virus in hatchery water supplies is essential to the future of broodstock culling. It is clear that both the horizontal and vertical modes of transmission must be controlled. What is less clear is the relative importance of each transmission mode at a given hatchery. Indeed, the relative importance of each may change from hatchery to hatchery. There are several well-documented reports of egg-associated (vertical) transmission of IHN virus. Most of these cases are based on isolations or outbreaks of IHN which occurred when salmonid eggs from the Pacific Northwest were sent to eastern parts of the United States, where IHN virus is not enzootic. However, the irrefutable demonstration of virus in a hatchery water supply serving as the source of infection for an outbreak of the disease has not been reported.

The differences in assay technique used for broodstock culling may account for the different mortality rates observed between Cowlitz and other hatcheries. This difference can be examined by using the infection rate and titer data determined for the Cowlitz Hatchery fish. The effects of testing pooled and diluted samples are related to the incidences and amounts of virus available. If eggs from five females are pooled, then a sample of ovarian fluid taken, and there is only one virus-positive fish per five-fish pool (a likley occurrence with the approximately 20% incidence at Cowlitz Hatchery),

there is, in effect, a 1:5 dilution of the virus. If this sample is then diluted 1:20, a very moderate amount, to avoid sample toxicity, the total dilution from the original is then 1:lOO (10^2). The effective cumulative dilution from the original using these methods is then l:lOO, meaning there must be a minimum of 10^3 infectious units in the original to get one infectious unit into a 0.1 ml inoculum. For example, in a five-fish pool there is one positive fish with 100 infectious units/ml, taking a pooled ovarian fluid sample results in a 1:5 dilution, so that only 20 infectious units/ml are present. A 1:20 dilution for toxicity avoidance means that only one infectious unit is presented to the detection system.

The minimum virus titer necessary for detection by the pooled fish/dilution method is 10^2 infectious units/ml (ignoring the additional factor of inoculum size). One can examine the titers determined for Cowlitz Hatchery fish by the single fish/no dilution method to judge how many fish might have been missed if the pooled fish/dilution method had been applied. As can be seen in Figure 3, there was a total of 58% of the cutthroat trout, 18% of the summer steelhead, and 24% of the winter steelhead with levels of virus below the calculated minimum amount. Of course, some carrier fish undoubtedly were missed with the single fish method, but presumably they would have much lower levels of virus in them than the fish that would be missed by the pooled fish method.

It is possible that the differences in mortality levels seen between the hatcheries whose fish were culled as a result of different assay methods are due to differences in the number of carrier females with low virus titers that were spared in the cell. It must be realized that this reasoning is based on logic and not an actual demonstration of differences in detection efficiency. Repeating the experiment at Cowlitz Hatchery and substituting the

single fish method at a second hatchery previously using the pooled fish method should resolve this discrepancy. Meanwhile, it is important for managers to avoid making irrevocable decisions based on assumptions of what happened at these hatcheries in the first year of broodstock culling. While complete understanding of the mechanisms involved in transmission of IHN virus may take years, sufficient information should be obtained within several breeding seasons to determine the future of broodstock culling as a control mechanism.

Potential role of carrier male fish in vertical transmission of IHN virus.

As practiced in the first year, broodstock culling ignored the possible role of male fish in the transmission of IHN virus. This was a conscious decision based on the need to reduce the workload involved in the culling process and on past observations of infection rates in males. The infection rates for males have appeared to be half those of females of the same populations, and levels of virus in individual males were a small fraction of those in females.

However, 6ome recent observations have cast doubt on the wisdom of ignoring the male fish's role in transmission of the virus. We have found that IHN virus adsorbs to salmonid sperm in a quantitative manner, with up to 99% of the virus removed from suspension within one minute (Figure 5). The attachment appears to be quite strong, but some recovery, at a low efficiency, can be made of adsorbed virus, indicating that the virus is not inactivated by the interaction with the sperm. Experiments on this phenomenon continue, but we can speculate that attachment of the virus to the sperm may represent a mechanism for active transport of the virus into the egg, with the sperm as the vehicle for such movement.

While the role of such attachment in transmission of the virus must be demonstrated experimentally, the implications of this mechanism must be dealt with in the broodstock culling efforts. Two questions arise: first, should males also be culled; and second, how should they be tested? Until it can be shown that male virus-carrier fish do not play a role in vertical transmission of the virus, they should'definitely be culled wherever feasible. ThiS assumption effectively doubles the work load and expense involved. Because of the strong attachment of the virus to the sperm, it may not be possible to merely test the milt from male fish for the presence of IHN virus. If 99% of the virus is attached to the sperm, there may be insufficient virus remaining in the seminal plasma for detection. Also, the strong attachment of the virus to the sperm may prevent infection of cell cultures, even if the sperm themselves are part of the inoculum. It may be that consideration6 such a6 these explain the observed low incidence and level of virus in milt samples. On a practical level, culling of carrier male fish will probably require testing of visceral organs for the virus, a significant increase in workload compared to testing of fluid samples which require no homogenization.

The role of male fish in vertical transmission of IHN virus is a high priority research area. If sperm do act as the vehicle for the entry of the virus into the egg, it is of special interest to determine the source of virus that the sperm carry. It is possible that the source of virus attached to the sperm is the virus released with the eggs in the ovarian fluid, rather than virus that is produced within the body of the male. If that is the case, it will be possible to again ignore the male fish and concentrate solely on the females.

Table 1. Incidence of IHN virus in sea-run cutthroat and steelhead trout tested as part of the 1981 broodyear broodstock culling experiment at Cowlitz Hatchery, and graded by length into groups based on the number of years spent in the ocean. Virus positive/virus negative. Percent incidence in parentheses. CTT= sea-run cutthroat trout, SST= summer steelhead trout, WST= winter steelhead trout.

	Number of Years in the Ocean			Total
	1	2	3	
CTT	9/68 (13%)	55/353 (6%)	5/47 (9%)	69/468 (15%)
SST		20/111 (18%)	9/42 (21%)	29/153 (19%)
WST		16/148 (11%)	86/320 (27%)	102/468 (22%)

Figure 1. Growth across a gradient of temperatures of five isolates of IHN virus obtained from hatchery and feral fish in California and Oregon. The straight horizontal line in each graph represents the amount of virus determined to be present in the cultures at the start of the experiment; data points located above that line represent true viral replication. CO- Coleman **National Fish Hatchery, ER= Elk River, TH= Trinity Hatchery, WS= Warm Springs Hatchery, NS= Nan-Scott Lake.**

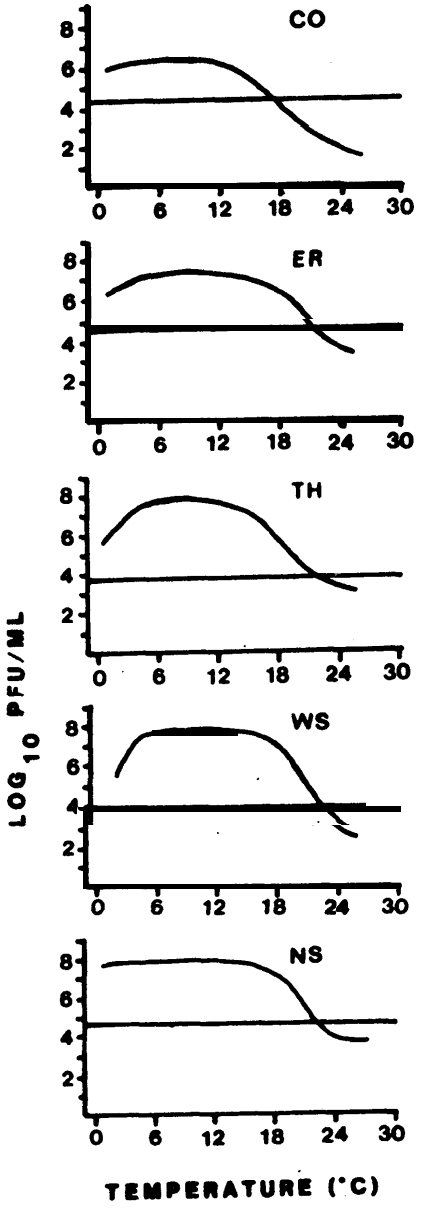


Figure 2. Incidence of IHN virus in returning female fish tested weekly in the 1981 broodyear broodstock culling experiment at Cowlitz Hatchery. CTT= sea-run cutthroat trout, SST= summer steelhead trout, WST- winter steelhead trout.

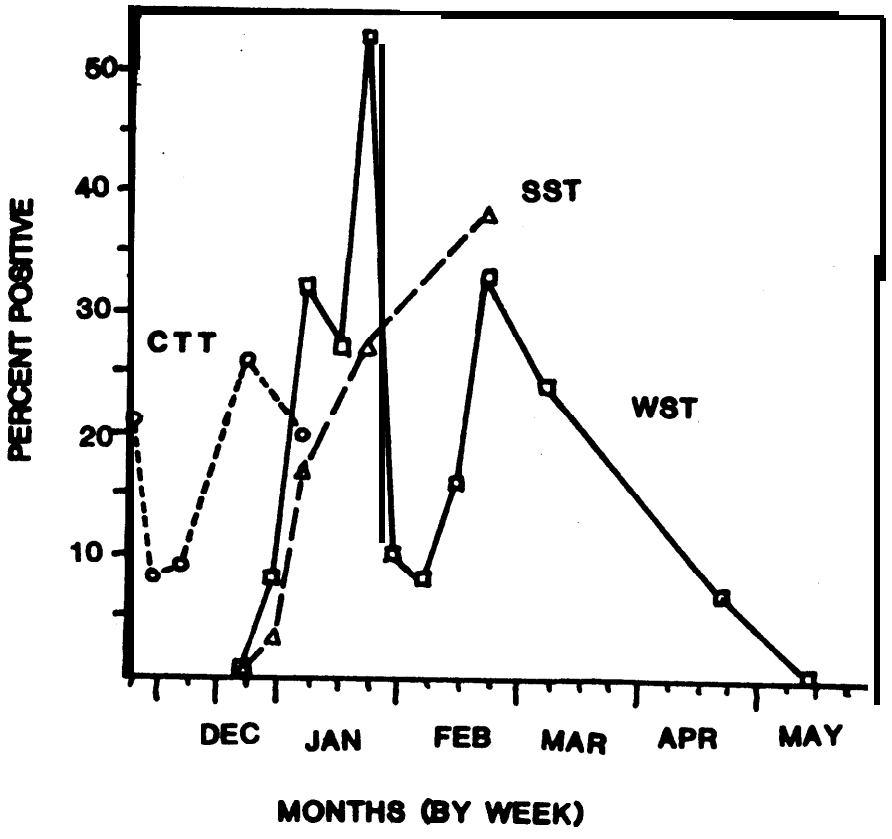


Figure 3. Distribution of viral titers obtained for individual fish of three populations at Cowlitz Steelhead Hatchery culled for IHN virus carriers in the 1981 broodyear. Number above each bar is the percent of all fish tested that fell within each interval of one $\log_{10}$ pfu/ml. CTT= sea-run cutthroat trout, SST- summer steelhead trout, WST- winter steelhead trout. Number below stock abbreviation is the overall viral incidence for that stock.

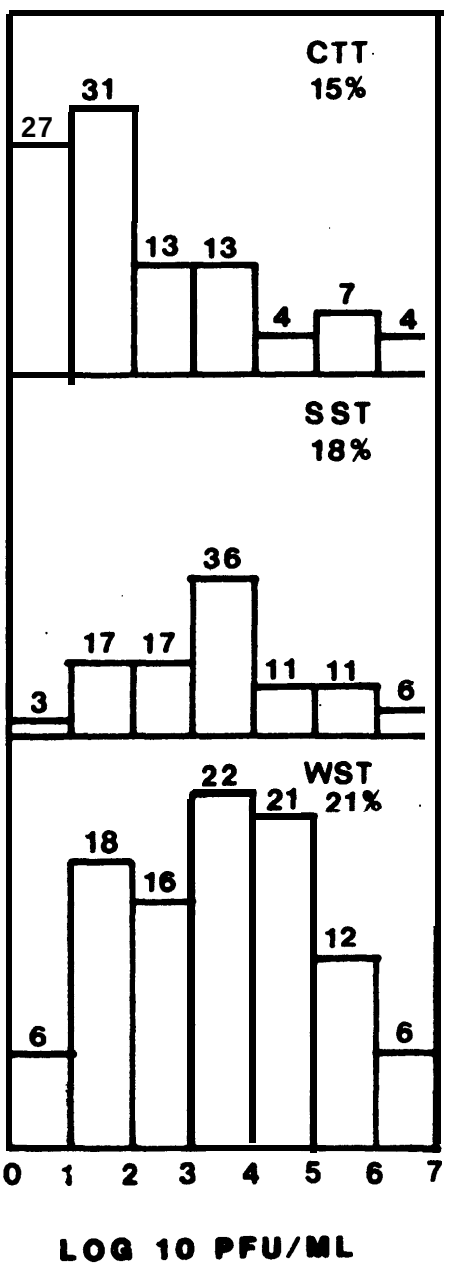


Figure 4. Weekly incidence of IHN virus in three stocks of fish graded by length according to the number of years spent in the ocean, in the 1981 broodstock culling experiments at Cowlitz Hatchery. The broad dotted'line indicates 1 ocean year; fine dotted line, 2 ocean years; and solid line, 3 ocean years. CTT- cutthroat trout, SST- summer steelhead trout, WST- winter steelhead trout.

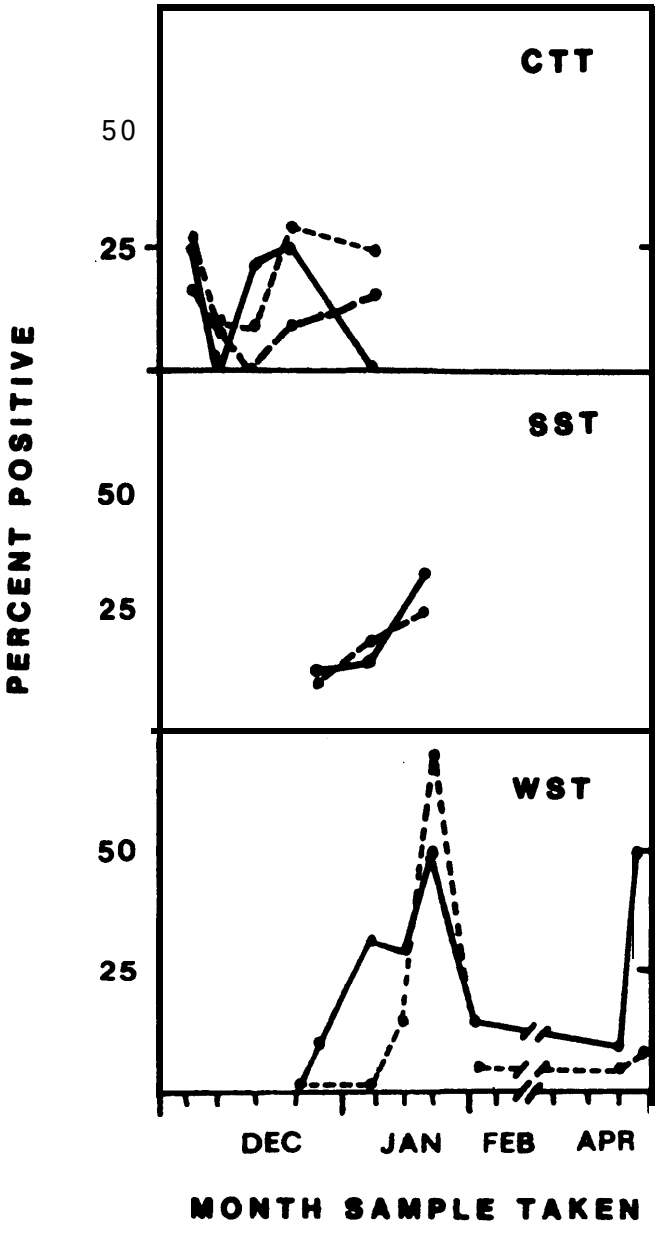
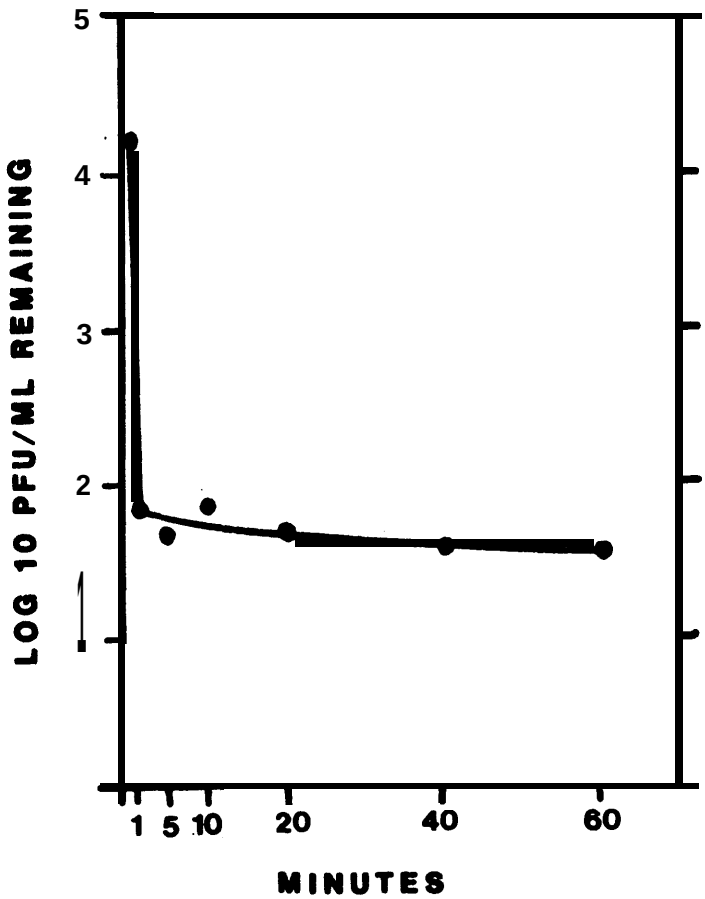


Figure 5. Decrease in IHN virus concentration in a suspension following addition of steelhead trout sperm at tim zero. Sperm and a known amount of virus were mixed, incubated at room temperature for the indicated time with gentle mixing. At each sample time, the sperm were removed by centrifugation and the virus remaining in the supematant was titrated.



Questions and Answers Following D. Mulcahy's Presentation

K. Amos Is there a correlation between the wild salmon populations you've tested and found low levels of IHN virus versus the Cedar River stock which is virtually a wild population, yet has high levels of virus?

D. Mulcahy A 'wild' population has evolved over thousands of years, not the thirty or so years since the Cedar River stock was introduced. The host-pathogen relationship in the Cedar River stock has been altered by the actions of man. The stock was placed in the Cedar River as a relatively small introduction. The Cedar River population has been the focus of our investigations for the last three years.

K. Amos Is it possible that the stock introduced into Cedar River was not infected prior to the introduction?

D. Mulcahy Every population of sockeye salmon has IHN virus. The situation in Cedar River population is severe, with an annual one hundred percent infection rate- and a very virulent virus. The yield per cell is tremendously high; it has the highest growth in vitro of any of the strains . we've tested. We've killed two-year old sockeye with water-borne challenge. There is an IHN epizootic every year at the Cedar River Hatchery.

K. Amos What was the method in which fish were trapped and held at the Cedar Hatchery?

D. Mulcahy Fish are trapped into small side channels with low water flow. There appears to be a slight increase in titer in the fish held in the side channels.

The most salient feature of the IHN virus cycle is that you can only find it for such a minute part of the life cycle of fish. In sockeye, it pops up within a few days of a fish's spawning. Even if they are held for months, the virus isn't there till they ripen up and spawn. It doesn't appear until such a time that it can't damage the reproductive processes of the host and it won't be eliminated by immunological processes. This speaks of an ancient host-pathogen relationship.

The primary directive for fish virus control is: don't make the situation worse by spreading the disease. Such could be the case if carrier fish are released, because no control procedures are available.

W. Groberg In human diseases, the first breakthrough was sanitation; the second was drugs and chemicals; and the third was vaccines. We aren't to the first step yet with fish, iet alone the third.

K. Amos Have you ever done water counts down in the Cowlitz River?

D. Mulcahy No. Without a concentration method, you're wasting your time.

K. Amos More specifically, have you tested water at the end of a raceway, or in the holding pond?

D. Mulcahy No, not at Cowlitz Hatchery, but I have isolated virus from water in the egg boxes at the Cedar River Hatchery. JoAnn Leong did it at Round Butte Hatchery and found four hundred infectious units/ml coming out of a Heath tray.

L. Ray At our hatchery, after the first IHN outbreak, we tried hatching egg8 and took a ninety-five percent loss. Since then, we've been buying fingerlings and bringing them in, from commercial hatcheries that have not had IHN, to my knowledge. Every lot we've brought onto the farm has come down with IHN.

D. Mulcahy What's your water supply?

L. Ray The same as the state hatchery at Hagerman, with a mile of a canal before it reaches us with lots of fish in it. Our losses ran from fifty percent on fish at five hundred per pound to fifteen percent for fish at eight per pound. But we've dropped that to about three percent. As soon as fish break with the disease, we cut the feed to fifteen to twenty percent of normal. With that procedure, our mortalities now run at two to three percent.

B. Busch Do you think that'8 due to reduction of stress or to feeding frenzy?

L. Ray Well, the first thing we did was to put in divider8 which forces water under the bottom. This improved water quality. Mortalities dropped in half. Then we reduced feed levels too.

D. Mulcahy You're agreeing with what I've been saying, that hatchery practice8 can contribute to reducing mortalities.

L. Ray Yes. Reduce stress and you reduce mortality. If fish are consuming less feed, the metabolic rate is slower and the demand on the fish is less. l don't believe we've brought a load of fish in, at two to three batches a month, that didn't come down with the disease.

D. Mulcahy I'd want to know, where did the fish come from, what checks were done, who did them, what cell lines were used, what dilutions --that's what I mean by "cast in iron" proof that the viral transmission was horizontal, rather than vertical. I don't want to see sixty fish grouped when you're bringing in eggs from five thousand adults. I want to see five hundred or one thousand fish looked at. It isn't enough to say fish were negative.

L. Ray Fish were comming from 54-56" F water and going into 58-60° F water.

W. Groberg How soon does mortality start dropping off after you stop feeding them?

L. Ray In three to five days, and then in two to three weeks it is over with. We've had to wait for as long as eight weeks before the disease would break after moving the fish in.

W. Groberg Viruses like healthy growing cells. Maybe you're just stopping the normal cell metabolism on which the virus depends.

D. Mulcahy It could very well be a nutritional factor. Classic treatment for several viruses is starvation.

L. Ray We've found that if fish weren't fed well before coming on the farm, mortality was much higher. Our supplier now feeds them well for the last thirty days before we get them. We continue to feed the well until the disease appears. As soon as we see a sign of the disease, we reduce the feed.

D. Mulcahy Is it a nutritional trigger, or is there a stage of development involved in this phenomenon? One of the earliest observations was that disease first struck the fattest, healthiest, best-looking fish.

The only successful control of IHN has been in California in the Sacramento River chinook hatcheries, where IHN mortality can be stopped after the epizootic begins, by raising the water temperature to about 57-58" F. Several other investigators have tried to repeat this with other species, and it hasn‘t worked. The reason for this is because the strain of virus in the Sacramento Valley is unique and is unusually temperature sensitive. It stops growing at a lower temperature than all other strains.

E. Wold Are you routinely checking males and females in your broodstock culling experiment?

D. Mulcahy Up until six months ago, we ignored males for two reasons: to cut the work-load somewhat; and because the infection rate and level of virus in females always was much higher than that found in males, by half. For years that bothered me, then I did an experiment: I threw virus in on sperm to see if virus could adhere to sperm. Not only does it adhere, but there's almost quantitative removal of the virus from suspension. Fish sperm binds IHN virus with amazing efficiency - greater than ninety percent of the virus, and in less than one minute.

If sperm is not stored properly, it will not adsorb sperm. Adsorption of IHN virus to sperm occurs using steelhead, rainbow, kokanee, and chinook sperm. This works for up to seven days after sperm has been taken from the fish, if it is stored at refrigerator temperature.

We will have to look at the role of other males further. It will more than double our workload in the broodstock culling experiment. The male's role in transmission of virus may turn out to be important from the standpoint of supplying the virus. The source of virus may be ovarian fluid and the male contributes the sperm as the carrier.

J. Rohovec In Japan, IHNV has been controlled by taking eggs from carriers to a clean water site, letting the fish grow up past the susceptible age, and then taking them back to te original hatchery.

D. Mulcahy How many years have passed without a reoccurrence? Is it that they don't have mortality, or have they eliminated it from the population?

.I. Rohovec They don't have mortality, or they may have a much lower mortality. I really don't know the specifics.

D. Mulcahy I'd like to see it done for ten years before conclusions are reached.

J. Rohovec We've taken eggs from virus-infected fish, brought them back to our fish disease lab which has a clean water source, and treated half with wescodyne and hoped that the other half would be positive controls. In neither group were there virus-infected fry.

D. Mulcahy There were two instances where we found IHN by bringing eggs into our laboratory. One case involved brining in green eggs, and in the second case, brining in eyed eggs, from a wild stock of naturally spawning fish from Lake Ozette, Olympic Peninsula. They vaccumed eggs from natural redds and brought four hundred eggs and we had actual mortality caused by IHN virus.

W. Groberg How reliable is your water source in terms of being virus free?

D. Mulcahy Chlorinated, de-chlorinated city water.

The Acquisition and Transfer of Fish Health Data

Kevin H. Amos

Washington Department of Fisheries

115 General Administration Building

Olympia, Washington 98504

I. Introduction

The imminent threat of viral diseases running rampant through our salmonid hatcheries is no longer a nightmare choreographed by fish virologists but is, indeed, a reality. As mentioned by previous speakers, IHN virus has caused severe health problems in lower Columbia River steelhead and salmon hatcheries, not to mention the losses that occur in private hatcheries that often go unreported. I think that of all infectious agents, IHNV poses the most serious threat to our salmonid resources in the Columbia River. For this reason, it is imperative that we try to control viral disease now and prevent their spread to new hatcheries and waterhseds.

In order to control any infectious disease, we must know where it is located, the virulence of the disease, the incidence, and the potential for the disease to spread. This information is pooled under the heading of epidemiology or the study of epidemics (in animals, the study of enzootics). Only after we understand the epidemiology of a given disease can we implement an effective erradication or control program. An integral component in determining the epidemiology is the acquisition, compilation and analysis of relevant data. An example of the importance placed upon gathering human health data is the system employed by doctors and public health officials in reporting the occurrence of certain diseases to the Center for Disease Control (CDC) in Atlanta, Georgia. Many millions are spent annually in this data collection and exchange process in order to help locate, prevent the spread and better understand the nature of certain infectious agents. The need exists for a similar program for viral diseases on the Columbia River watershed.

II. Current Methods

Fish virological data is compiled primarily by biologists who conduct disease inspections and certifications. There are less than a dozen laboratories which routinely examine fish samples from the Columbia watershed for virus. The records maintained may range in sophistication from a log entry to a detailed health report and history entered into a computer. Typically, the former is of most common use.

There is no formal system established for the exchange of information between pathologists. Details relating to a viral epidemic are often passed unofficially by word of mouth between virologists. The current method is inefficient and often has the result of passing incomplete and inaccurate information. Another problem associated with exchanging data relating to a viral isolation is that organizations, private or government, feel that it is of no other person's business as to what disease problems are occurring at their facilities. The guilty party is often afraid of undesirable repercussions; however, this Victorian attitude is often the cause of the problem Increasing in severity.

On a positive note, primarily due to the IHN problems in the lower Columbia, virologists in Washington, Oregon and Idaho have started working together and exchanging information. Last year, Dr. Warren Groberg started compiling data on all viral epizootics in the Columbia drainage. Unfortunately, the picture is incomplete in that not all hatcheries in the three states participated in the survey. In addition, we only have a few years of historic data on most stocks available to us for analysis.

III. Proposed Methods

We are all aware that in order to understand and control a disease it is imperative to have a complete health picture and history of the animal it’s

affecting, To control IHN, therefore, we must establish a data collection system which can efficiently and accurately portray the current and historic status of viral disease6 in all Columbia River watershed hatcheries. There are other diseases beside6 IHN and IPN which also should be monitored in some manner. However, we will consider only these particular agents.

We must first determine what information is desired for a health history. The following items are of paramount importance: 1) the location of the fish, 2) identification by species, stock and age, 3) current health problems of fish sampled (to include mortality and carrier incidence), 4) health history of the stock, and 5) disease history of the hatchery and watersheds to which the fish have been exposed. The preceeding data should be collected and maintained at a central location on a routine basis. As part of the implementation of the new State of Washington Fish Disease Policy it will be necessary for the two state agencies to maintain, on file, a current health picture of all of their hatcheries, to include the status of certain viral, bacterial and parasitic agents. This information will be readily available to all interested parties. I would recommend that this procedure be followed by all three states, especially for viral diseases in order to establish the current disease status in their respective stocks and facilities. Here is an example of the form now in use by the Washington Department of Fisheries. (Form attached)

Second, we must have a system of reporting new isolations or epizootics. I would propose a system similar to that of the Public Health Offices. Upon the discovery of certain reportable diseases (in our case, IHN or IPN), the pathologist or hatchery biologist would notify a designated state virologist and inform them of the particulars of the isolation. This notification should be in writing as well as orally. The state pathologist

would then inform in writing, a laboratory or individual who would be responsible for collecting and collating the disease information in a manner so a6 to make the data readily available to other pathologists or researchers.

A final consideration in the data collection and transfer process is the type of technology to be used. For the initial transfer of information from the hatchery biologist to the state virologist, I would recommend a form similar to the one we've already discussed. The method of transfer and storage of the vital statistics from the state virologist to a centralized location lends itself to the use of computers. A system could be used in which each state virologist has a terminal to a centralized computer and could record or retrieve relevant disease data. The pathologist for Fisheries and Oceans in British Columbia, Gary Hoskins, utilizes a Univac System 2000, located in Victoria to record all his health histories. He sends and retrieves information from his terminal in Nanaimo. Another possibility for information storage would be for the state virologist to simply pass on a copy of the disease history he receives directly to one centralized terminal, which would have the primary responsibility for introduction into a computer. In either case, the most efficient method for storage and retrieval of data is by the use of a computer. Many commercial programs currently exist which easily could be adapted to our needs.

Once we have complete viral histories of all Columbia Basin hatcheries and stocks readily available, we will have a multi-faceted tool to help us control IHN. The available data can be used to prevent dissemination of IHN, elucidate how the disease spreads, and possibly where the disease may exist next. These are just a few of the jobs that a centralized storage system could help us accomplish.

In conclusion, I would like to point out that in attacking the IHN problem a well thought out and coordinated effort must be implemented in order to use resource6 efficiently and expeditiously. What we are fostering today is exactly what is needed to give us our best chance in controlling a disease which potentially could jeopardize the economic feasibility of rearing anadromous salmonids in the Columbia River.

Questions and Answers Following K. Amos's Presentation

E. Wold — National Marine Fisheries Service (NMFS) provides funds for 22 hatcheries. Starting October 1, 1982, we will include the requirement for data collection, disease history, occurrences and other cost factors involved in hatcheries to determine cost of rearing fish over a period of time - from two hundred per pound to smolt size. Part of collection will be losses due to specific disease - cause, amount of loss, and cost in'lost production. We do have collection forms that hatchery operations will be using. We'll have a centralized computer in the office and cassettes in various locations.

D. Mulcahy — This is critical for an assessment of the situation. Is it getting better or worse? We need quantitative analysis.

J. Leong — What authority backs the Washington Fish Disease Policy?

K. Amos — (Reading from form) A Washington Administrative Code (WAC 220-20-039). It is law authorized by the Director of the Agency, as opposed to Washington State Code which is authorized by the State Legislature.

J. Leong — But is it enforceable?

K. Amos — Yes. An administrative code (WAC) is a state law and is enforceable. There is, however, a question of jurisdiction in regard to Indian tribes and watersheds on tribal property. Washington has jurisdiction over all watersheds (Federal or otherwise) in the state. So in the case of a Federal hatchery on a state river, the state has authority over what is dumped or planted in the river. However, if a coastal tribe's reservation includes part of the coast line, the state has questionable authority on disease matters regarding hatchery releases. Our intention is to foster cooperation, not to arrest people. The Disease Policy has formalized the process of making a request for more fish and has defined what is and is not allowed.

D. Mulcahy — It is not good to connect the permitting process and law with the information gathering process because of suspiciions that if they donate information to a central place, a regulator is going to show up to tell them what fish they'll have to kill and sell, and what to do with them. This is not the idea at all.

K. Amos — Right. The Disease Policy does not give the State the authority to go in and destroy anybody's fish unless they have VHS (Viral Hemorrhagic Septicemia virus) or Myxosoma cerabralis in their hatchery.

W. Brunson Originally, the committee formulating this policy was composed of representatives from all concerned people.

K. Amos Right. The Disease Policy Committee had input into the content of the policy, but as a group had no authority for making the policy a law.

W. Brunson We have authority to regulate fish in and out of the state and within the state as well.

Viral Disease Considerations in the Commercial Trout Industry in Idaho

Robert A. Busch

Clear Springs Trout Company

P.O. Box 712

Buhl, Idaho 83316

Introduction

It is necessary to have an understanding of the commercial trout industry and where it came from as there are some important and basic differences between fish health management and particularly virus disease control at a commercial trout hatchery in Idaho as compared to a resource or mitigation hatchery elsewhere in the Columbia River Basin.

The Idaho industry began raising fish for live haul and stocking prior to World War II. This was at a time when none of us knew much about fish diseases or infectious disease processes in trout. Eggs and fish stocks were being moved around quite freely all over the U.S. and the world without any consideration being given to infectious diseases. Even in the 1940's and 1950's Idaho was already a recognized center of fish culture in North America. Consequently a lot of stocks were being moved in and out of the area by federal and state agencies as well as private industry. By the 1950's, many infectious diseases of trout had established endemic loci of infections in southern Idaho. In terms of certifiable diseases, we were already looking at infectious pancreatic necrosis (IPN) virus, enteric red mouth disease (*Yersinia ruckeri*), and furunculosis (*Aeromonas salmonicida*).

As we began to learn more about these diseases, in the late 1950's and early 1960's, we started seeing the establishment of some fish health control regulations , particularly at the state level. This combination of factors changed the face of the Idaho trout industry. California had been the largest market for live trout being hauled out of Idaho, Due to fish disease control regulations, that market was no longer available for live fish and forced the

industry to change its mode of operation to a processed food fish industry.. Consequently, today, 99% of the fish coming into the industry as eyed eggs are eventually processed as food fish. Today, only a very small percentage of our production is ever sold and shipped as live fish.

In addition, we have a couple of other unique features that differentiate us from typical resource or mitigation hatcheries: a constant, year-round water temperature of 14.5" (58°F); continuous hatchery production twelve months out of every year (we no longer have a season when inventories are up or down, we are in full production year round). This single factor becomes very critical when we begin to talk about practical and cost effective methods of viral disease control or eradication in commercial trout hatcheries.

At this point in time, the Idaho trout industry produces in excess of 90% of all the commercial rainbow trout in the U.S. We produce about 30 to 35 million pounds annually, and hatch about 80 to 100 million eggs/year. Some of the largest fish hatcheries in the world are in southern Idaho. We have one hatchery with a projected production capacity of 7 million pounds annually. That's more production from a single hatchery than most states produce all together.

The design of the industry is rather different compared to a typical mitigation hatchery'operation in the fact that we are a vertically integrated industry. The Idaho trout industry is dominated by four privately held companies. These operations maintain their own brood stock and egg production, their own feed mills and feed production, their own hatcheries,

their own processing plants, their own packaging and their own distribution. They own and control the companies completely from top to bottom. The one exception to this practice are the "farm pond" operations. These are small private production operations used periodically on a contract basis. A large producer may move a small lot of fish out to a small operator to grow-up to market size and then bring them back in for processing. However, for the most part, operations are closely controlled from top to bottom.

Stress and the economic impact of disease losses associated with it, is an important consideration in our operations. Our loading density and production has been going up every year. Average loading densities now are approaching 2 pounds per cubic foot of rearing space and can exceed that at times. Annual production at the newer installations is close to 20,000 pounds per cubic foot per second (CFS) of water flow. These factors result in high levels of stress on our fish that is present on a year-round continuous basis. Our hatcheries can be at their maximum stress levels every month of the year. Consequently, most infectious diseases, which are more or less stress-mediated, are major factors for us. This includes not only the endemic certifiable pathogens such as IPN virus, Y. ruckeri, Aeromonas salmonicida but also such ubiquitous diseases as myxobacterial gill disease.

Parasitic diseases are usually not much of a problem in our operations, primarily because of the rapid water turnover times in our ponds (4-6 times an hour). At the resulting high velocities, parasites can not establish themselves in most instances. However, we can have Sanguinicola, Salmonicola, and various protozoan and metazoan parasites, at times, but for the most part they are not a problem.

Contrary to popular belief, the Idaho commercial trout industry has not seen the introduction of a new pathogenic disease agent in 20 years, until IHN virus first appeared in 1977, and proliferative kidney disease (PKD) in 1981. This fact alone is strong testimony to the fact that the Idaho commercial trout industry is fully able to protect itself from the introduction of new infectious disease agents in the absence of formal regulation. Basically, by the design of our operations, our disease exposure is quite minimal. We have control over our egg supply which is brought in from certified hatcheries, and our water supplies are generally free of migrating wild or planted fish stocks. In the past 5-10 years, very few live fish have been hauled into the valley and virtually all of the eggs coming into Idaho are now certified as being disease-free. [1] *

History of Viral Diseases in Southern Idaho

IPN virus disease was the primary and sole endemic disease in the commercial trout industry of southern Idaho for many years. It was first recognized to be endemic to the upper Snake River drainage of southern Idaho (the Hagerman Valley as it is commonly called) in the early 1960's, and was quite likely present prior to that. Prior to the appearance of IHN virus in 1977, IPN virus was considered endemic to all hatcheries in the valley and was maintained through horizontal tramsmission within the hatchery. Disease-free eggs were brought in to the hatchery buildings and maintained on spring water

* Numbers correspond to questions raised during presentation. Refer to numbered comments in "Questions and Answers" section.

supplies, which tend to be disease-free for all practical purposes. There is very little disease of any kind in the hatchery buildings themselves as the troughs are routinely cropped, de-watered, and disinfected between production lots of fish. However, most commercial trout hatcheries insouthern Idaho are not able to adequately disinfect ponds outside of their hatchery buildings, due to inherent design and management considerations. Consequently, once the virus-free fry fish are moved to the outside ponds, they are exposed to virus infection. Characteristically, fry fish go to the outside ponds at about a 0.5-4.0 gram size, depending on the season of the year. Within 7-14 days they commonly broke with acute IPN virus disease. Mortality ran from 10-80%, an average for IPN losses being 25% of the fish ponded. No recurrence of viral mortality was seen following recovery from the initial infection but continuous chronic infection and shedding of the virus in the feces occurs. IPN virus was easily isolated from virtually any stock of fish in the Hagerman Valley. However, the establishment of an endemic infection of IHN virus in 1977 drastically changed the ecology and epizootiology of IPN virus as just described.

IHN virus has been previously isolated from rainbow trout in many areas of the United States including the Hagerman Valley of southern Idaho but the clinical condition has always been associated with a chronic low virulence infection that did not develop to an endemic condition. The first documented isolation of this "new" highly virulent strain of IHN virus in the Hagerman Valley of southern Idaho was in January of 1977. In that year it was isolated at two separate hatcheries, one in January, and one in February. These two hatcheries were very much isolated from each other with no common exposure of trucks, equipment or personnel. After the initial isolations in January and

February 1977, IHN virus was not seen again for 10 months, until January 1978 when three more outbreaks were diagnosed. From January 1978 and for the next two years IHN virus proceeded to spread throughout the Hagerman Valley to eventually involve virtually every hatchery facility in the area.

Due to the inherent design and management of commercial trout hatcheries, there is a great deal of movement of fish stocks within a given hatchery operation or company. Clear Springs Trout Company, for instance, has five hatcheries' and routinely moves stocks between those facilities. However, our trucks, equipment, personnel, and fish never come in contact with another hatcheries operations. So, even though there is a great deal of movement, it is almost exclusively within a given company and operation.

In a matter of just three years IHN virus spread throughout the Valley and became endemic in virtually every operation there. Evidently, this "new" strain of the virus is a highly virulent pathogen for rainbow trout at **14.5°C,** and is very easily transmitted within and between typical hatchery operations. I think we would all agree that one of the more logical considerations in determining the possible reasons for the recent rapid dissemination of IHN virus in the Columbia River Basin is the movement and straying of anadromous stocks. We have an area in southern Idaho, in the Hager-man Valley, where IHN is now endemic. In this endemic foci, we already have one steelhead mitigation hatchery, Niagara Springs, which has a history of IHN The Hagerman National Fish Hatchery is presently under re-construction and is to be turned into a steelhead mitigation hatchery. And the U.S. Army Corps of Engineers has purchased Crystal Springs Ranch hatchery as yet a third steelhead hatchery in this area. I cannot help but question if

real serious consideration has been given to the simple fact that siting additional anadromous mitigation hatcheries in an endemically infected area as concentrated as the Hagerman Valley has to be biologically unsound at best.[2]

Ecology and Epidemiology in Hatchery Populations

The incidence of occurrence of IHN virus in the Hagerman Valley is very interesting. The epidemiology of the disease is a classic study of the introduction of a virulent new virus into a naive, susceptible stock of fish concentrated in a small geographical area, When IHN was first diagnosed in 1977 and 1978, it appeared as a chronic infection of large rainbow trout anywhere from 100-500 grams in size. Chronic mortality typically extended for 6-7 weeks. Gross clinical signs were characterized by general lethargy and quite a bit of what appeared to be neuromuscular involvement as infected populations were very excitable. Death was very slow and often fish would be on the tail screens for several days before they would actually die. Total cumulative mortality during the clinical course of the disease was typically around 25 percent.

Clinical examination indicated only a moderate anemia. The kidney and gills were not particularly pale. The infected populations typically appeared to be chronically debilitated and secondary infections were common. Bacterial gill disease, enteric redmouth, and furunculosis, *were* all common secondary pathogens and contributed significantly to overall mortality. Even systemic Aeromonas and Pseudomonus infections that are not usually found in these stocks became quite common indicating that the host resistance was definitely compromised. A rather interesting secondary systemic myxobacterial-type infection predominant in the spleen and kidney was also noted.

Following the initial appearance of IHN virus as a chronic infection of large fish in 1977 and 1978, subsequent years show intial infection beginning in progressively smaller fish and clinical course of infection become more acute. The disease literally moved up within an infected-hatchery operation from large market fish ponds on the bottom up into fry and fingerling ponds and finally into the hatchery buildings themselves. At the present time, IHN virus in rainbow trout in the Hagerman Valley most commonly occurs 8-10 days after ponding fish outside of the hatchery building. In these l-5 gram fish, mortality peaks in 10-14 days and averages as high as 70 percent mortality in some operations. Overall in the industry at this time, average IHN losses would be around 30 percent.

A logical explanation for the present situation is that water supplies are functionally free of the virus, hatchery buildings are adequately disinfected and free of the virus, egg supplies are certified free of the virus, and consequently, as long as fish are maintained in the hatchery buildings under controlled conditions they also remain free of the virus. However, it is usually not possible to properly disinfect outside ponds between production lots of fish so that once virus free fingerlings are ponded outside, they become infected and undergo significant mortality. There is no evidence that the disease is recurrent so that morality is no longer seen in large fish. The only time IHN virus disease losses are taken in large fish at the present time is when they have not been previously exposed to the virus.

As both IPN and IHN virus are now both endemic to the Hagerman Valley, some interesting observations can be made upon their relationships with one another. When IHN virus first appeared in 1977-78-79 and IPN virus was already endemic to our operations, the fingerling fish would initially be free of any virus at the time that they were first ponded outside. However, soon after ponding outside, they would become.infected with IPN virus and undergo an IPN epizootic, with approximately 25% mortality incurred. Survivors would then be moved down through the operation and as they were moved into the lower production ponds, they would become infected with IHN virus and suffer a chronic low-level mortality of approximately 20 percent. At this time both acute IHN and a carrier incidence of IPN virus could be isolated on tissue culture.

In 1979-80, when IHN virus losses began to occur in smaller fish they would still be ponded outside in a virus free condition, first develop an IPN virus infection and then an IHN virus infection. The only difference was mortality to the IHN virus began to increase. Instead of a chronic infection with 20 percent mortality an acute or sub-acute infection, with 30 to 50 percent was common. At this time, both IHN and IPN virus could be isolated from moribund fish on tissue culture and histopathological examination often indicated a concurrent infection.

As IHN virus began to appear in fry and fingerling fish in the top fry ponds or even in the hatchery buildings in 1980-82, prior to contact with the endemic IPN virus, only the acute to peracute form of IHN virus developed and IPN virus was no longer isolated, not even as a "carrier" type of'infection. There is some type of an interference mechanism established by IHN that

precludes suprainfection with IPN virus at a later time. Approximately 70 percent of the hatcheries in the Hagerman Valley are at the stage where there is no IPN isolated and IHN is the only virus found.

Ecology and Epidemiology in Broodstock Operations

A final consideration are observations made on the affect of accidental IHN virus introduction into a certified virus free broodstock population of rainbow trout situated in a geographically isolated location away from the commercial industry and operating on cold (52°F) artesian well water.

A particular stock of fish in the Hagerman Valley was wanted for broodstock. They had been checked for virus, but nobody had bothered to explain that IHN virus cannot be isolated from the asymptomatic carrier state of infection. Assuming them to be free of all viruses including IHN, they were moved into one of the broodstock facilities. This occurrence, unfortunately, presented an excellent opportunity to examine the epidemiology of this disease in a naive population of rainbow trout broodstock at 52°F.

It was a very old and well established broodstock operation that had been routinely inspected and had absolutely no prior history of IHN virus. Two years ago, this stock carrying IHN virus as a latent infection, was introduced into the broodstations as yearlings and were raised to two year olds. As sexually mature two year olds they were moved from holding ponds to the main spawning facility where the eggs were spawned, fertilized, and washed. The effluent water from the spawning facility went directly into one end of the head ditch feeding the main spawning facility. It was from these eggs that we got our first outbreak of IHN. It was a peracute outbreak, the likes of which

we had never experienced before. It resulted in yet a third peracute type, or clinical appearance, of IHN virus that we now feel is typical of vertical transmission. That is, mortality occurred in sac-fry or swim-up fry right in the incubators and 95 percent to 99 percent loss occurred in 24 to 72 hours.

In addition, it was theorized and later proven that as the washings from those eggs went down into the head ditch and over the rest of the broodstock including 3, 4, 5, 6 and 7 year old fish, the virus was horizontally transmitted to these stocks. We saw no mortality in the newly infected broodstock but all egg takes the following year form these fish were positive for IHN virus. IHN virus titers in the ovarian fluids were anywhere from $10^5 - 10^8$ $TCID_{50}$/ml. This situation demonstrated to our satisfaction that this "new" strain of IHN virus is a highly infectious and virulent pathogen of rainbow trout and is readily transmitted by either vertical or horizontal means. [3]

Clinical Diagnosis

Rather than review the clinical signs of IHN virus in rainbow trout that we are all familiar with, let me make a few unique and interesting observations. Some fish exhibit a fecal cast, but it occurs only 20 percent of the time and is not considered pathonomonic in trout.

Another interesting observation is often made about 4 or 5 days before the onset of mortality when an obvious erosion of the dorsal fin appears. When examined histologically, this lesion is characterized by typical necrotic

destruction of the reticuloendothelial elements and a breakdown of the capillary beds. We do not know what the correlation is but it is a very consistent sign.

Still yet another interesting observation that we are making in rainbow trout goes back to some of the original descriptions of IHN virus made by Amend in sockeye salmon where he referred to the development of scoliosis in survivors. This same condition develops in rainbow trout. The incidence of scoliosis following an IHN epizootic in rainbow trout is anywhere from 2-4%. As these fish are not cosmetically acceptable for packaging, they are discarded. If we talk about 4% of 35 million pounds/year at $1,65/pound, this is a major problem. As a matter. of fact, in terms of strict economics, scoliosis may well be a bigger problem for the industry than the mortality itself.

Diagnosis

In terms of our diagnostic procedure, it is primarily the same microculture screen that I described in Seattle in 1980. We run two different dilutions and four different replicates of each pool of kidney, spleen and pyloric cecae. All of our tissues are run at 1:lOO and 1:200 dilutions, and our ovarian fluids are run at 1:20 and 1:40 dilutions.

Most of our diagnostic work is run on the EPC and CHSE cell lines. To speed up the whole process when IHN is strongly suspected, we will run serum neutralization tests at the same time as our primary screen. This is in situations where we are dealing with an epizootic ovarian fluid where the titers are high enough to demonstrate neutralization directly. This short cut

is particularly important in terms of our broodstock throw-out system. We have a race in running our viral screens, and serum neutralization before the eggs eye up and come down into the hatchery building. By running serum neutralization along with the screens in the microculture system we can often speed up the whole process.

As far as the histopathology is concerned, I feel it is necessary for a proper differential diagnosis, particularly if there is any chance of a mixed infection between IHN and IPN and to properly differentiate the primary pathogen.

Control Methods

In terms of control, prevention is the first concern. We have certified virus free egg supply, water supply and we maintain our hatchery buildings in a virus free state. The vast majority of our eggs are coming in from certified disease free stocks and they have never presented a problem. In the one instance where we do have demonstrated viral infection in a broodstock, we have established a throw-out type program. As we do not have the logistics or resources to do the volume of sample desired, we limit our system to sample 10% of all fish spawned, both male and female, as five fish pools. A group of five females are spawned, and an ovarian fluid sample is taken from the pool. They are then fertilized with a pool of males and a second sample is taken from those males. All pools are kept separate. The eggs are incubated and the samples come down to the lab for processing. Prior to the shipment of the eyed eggs down into the hatchery, they are sorted out on the basis of the viral results. All of the pools of eggs found to be infected with virus are

destroyed. All of the broodstock associated with those infected pools are also destroyed. Only egg lots found to be free of virus are brought down to the hatcheries for hatching and only those adult parents are maintained in the broodstock operation for future spawning.

Using this throw out system, we had a perfect record until three or four weeks ago when we did have an outbreak in an incubator. We were able to control it very easily and completely disinfect the system. Otherwise, we have been effectively able to stop any further introduction of virus from that broodstock into our production hatcheries.

In terms of disinfection of the hatchery as a whole, individual ponds, or the water supplies, 'this is relative to the hatchery design and location. Considering our water supplies can not be shut off (these are springs flowing at 350 cfs and cannot be turned off or dried up) and that, in many instances there are native stocks in the water supplies that cannot be removed. It is generally difficult or impossible to properly disinfect our water supplies. [4]

In terms of management as a control of IHN virus, I will admit that cutting back on feed to 25 percent of the normal ration is a standard practice in the Valley. The primary reason for this, of course, is that diseased fish go off their feed. But, in general, managers feel some degree of starvation reduces the mortality. I have not seen the hard figures that would convince me that it is an effective practice. I have not seen any difference in cutting fish back to starvation level as compared to cutting fish back to the amount that they are going off feed anyhow.

Probably the biggest single management control factor is stress. Most of these diseases including IHN virus are, to a large extent, stress mediated. In many instances we are able to significantly reduce mortality from 70 percent down to 30 percent simply by reducing stress. This may include ponding at very low densities, avoiding excessive handling or grading, going to demand type feeders, or keeping them on top water. By reducing stress, we are able to control losses to a very large measure. Conversely, anytime IHN virus infected fish are stressed, you can expect to take an excessive mortality.

In terms of control, another potential consideration is vaccines. We have already discussed some of the theoretical concerns with their potential use but if a live modified virus vaccine has significant potential for reducing mortality, it will be difficult to convince the commercial industry that they should not be using it. As you well know, live modified vaccines have already been field tested for IPN virus and IHN products are being looked at. However, before any viral vaccine products become effective, we are going to have to gain a better understanding of the protective mechanisms involved. Why do we not see the development of any signficant serum neutralizing factor following IHN epizootics? Working with Dr. Phil McAllister in Leetown, we have taken IHN infected populations and followed them from prior to infection through the epizootic and on to final market size by sampling every 30 days for a year. The only increase in serum neutralizing activity was a slight very transient increase just after recovery. But this factor is far from explaining the life long resistance to recurrent infection that is observed.5

Anti-viral chemotherapeutants are another consideration for cost effective disease control in fish. We have been working on various candidates for eight years now and have one that we have carried all the way through field trials. This particular chemotherapeutant, when administered in the feed, will stop IHN infections in rainbow trout with zero mortality, while paired control population8 suffer 70 percent mortality. However, when the drug is discontinued mortality resumes unabaited, The whole theory behind such a chemotheropeutant is to hold back the infection long enough to let the protective mechanism, whatever it is, develop. I have held it off for as much as 45 days with chemotherapeutants but seven days after the drug was discontinued, full mortality insued. I do not know what the protective factors are or how they develop. Will a vaccine, either live modified or killed, do the job? It appears that some type of latent infection must be developed in order to impart protection. [6]

Questions and Answers Following R. Busch's Presentation

1/ D. Mulcahy Who does most of the certification work?

B. Busch 'To the best of my knowledge, of the two largest egg suppliers, Mount Lassen Trout Farms in California is certified by the State of California and McLeary's Trout Lodge Hatchery in Washington, I believe, is now certified by McLeary himself; Skane Trout Farm in Washington is done by the U.S. Fish and Wildlife Service. Commercial broodstocks in Idaho are done by their own laboratories. With some exceptions, these are all certified disease-free stocks that have a long history of being specific pathogen free.

2/ W. Groberg On those throw out experiments, were the eggs iodophored?

B. Busch Yes.

w. Groberg Let's hypothesize that that was breaking the cycle as much as the throw-out was.

B. Busch Yes, but our eggs have always been iodophored, even prior to the introduction of IHN virus.

W. Groberg We hear from Japan that they eliminated transmission with iodophored eggs and this even included eggs from positive parents. It could still be the iodophore treatment in your case.

B. Busch It could be a contributing factor.

L. Ray On the throw-out plan, you sample ten percent of the fish?

B. Busch Every tenth fish that is spawned is sampled.

K. Amos So you are just surveying the broodstock?

W. Groberg So you know that out of the other ninety you may have some positives?

B. Busch When any fish in a pond is found to be positive for IHN then they all are considered to be positive and discarded.

D. Mulcahy What kind of positive sample rate are you getting?

B. Busch Generally about eight to ten percent of those in the infected plume

L. Ray Are they trying to eliminate that little triangle?

B. Busch Yes. Eggs are buried and brood stock is destroyed.

L. Ray What are your densities?

B. Busch Two pounds per cubic foot of rearing space.

L. Ray Flow?

B. Busch Twenty thousand pounds per cubic foot per second annually.

W. Groberg You say you have not seen IPN for eighteen months. Is that loss?

B. Busch There are still hatcheries where I know IPN virus occurs. But the hatcheries I have dealt with, that historically have had IPN and have been endemically infected for many years, and the same hatcheries where we have had mixed infections in the past, in the last eighteen months I have not had a single isolation of IPN.

D. Mulcahy I just picked up a double infection last week.

J. Rohovec A guess why?

B. Busch I think if you did challenge experiments in the'lab and the fish are exposed to IPN virus prior to IHN virus or even concurrently, you will get a mixed infection. However, if they are exposed to IHN virus initially, before the IPN virus and undergo an IHN epizootic, I think you will see an interference type phenomenon and they will not become infected with IPN. This is an hypothesis.

K. Amos In your first'identifications in 1977, have you been able to trace the possible source?

B. Busch Yes.

K. Amos Was the IHN problem at Niagara before or after IHN problems at other facilities? What year was it first found?

J. Rohovec Around 1977-78, I am not sure.

W. Groberg It was seen in Niagara in July 1978. How do you account for the fact that it went from two places to virtually every hatchery in the Valley in one year? Is it the bird theory?

B. Busch. I do not know. It happened too fast to blame it solely on sanitation. A lot of fish are moved, but the operations themselves are fairly isolated and it is just too fast to blame solely on sanitation.

W. Groberg That is almost what happened in the Basin in 1981, on a smaller scale.

B. Busch That is why I said there's got to be more to the transmission than merely sanitation.

W. Groberg Yes, something very complex.

D. Mulcahy All these weird things we can pick out that do not fit anything we know.

B. Busch Or contradict something we think we do know.

D. Mulcahy There are rules and an order to this thing, we just do not know what they are.

W. Groberg When I first started out, I believed everything I read. Now I am skeptical about many things I originally learned about. For example, I do not know if fish that survive an epizootic are carriers. I have a feeling they are, but I would like to see good experimental data to prove it.

D. Mulcahy Skepticism is a healthy viewpoint.

B. Busch An obvious question, in light of our discussion to this point, is this "new" strain of IHN virus basically the same organism as that which has long been recognized and studied in Pacific salmon? A lot of data has been presented that shows there are major significant difference between isolates of IHN virus.

E. Wold Any connection between the incidence of IHN and the use of grow-out ponds?

B. Busch No, not that I have been able to see.

We've got some pretty good evidence, that when suspect IHN virus infected eggs that are maintained for replacement brood stock, fry, fingerlings and so forth at 52°F at brood stations we do not see the IHN virus disease develop. However, there are a couple examples where it appears that stocks of fish hatched and raised to fingerlings in cold water and then brought down to the warm water in the Valley, have broke with virus. There is of course some question as to their disease state when they first came down. Whether this loss is associated with temperature, stress, or other factors, we do not know but we're interested in the role temperature may play in the development of the disease and subsequent protection in rainbow trout.

J. Rohovec What is the water source for the cold water?

B. Busch The same source as the production hatcheries only higher up in the aquifer and colder.

K. Amos Have you been surveying the fish above your intake, at Box Canyon Springs, and have they been negative up until this point?

B. Busch Yes, we have periodically sampled these stocks and could not isolate IHN virus.

K. Amos Have you done any surveillance on outfalls from your hatcheries at Box Canyon or Clear Springs?

B. Busch No.

K. Amos One might assume they are infected considering how it is passed down. Do you think you will be doing any kind of that work?

B. Busch We do not anticipate doing that type of work at this time.

K. Amos Our observation of clinical IHN in larger rainbow was the same as yours. At Cowlitz Hatchery, IHN was found first in the smaller steelhead fry. Then, the legal sized rainbow in the re-use water became infected with it.

W. Groberg We have had mortality in previous years but it has been so masked with ceratomyxa that it is difficult. . . I have never seen mortality like with IHN from ceratomyxa.

K. Amos I am trying to point out that the first exposure the legal-sized rainbow probably had was last year via steelhead fry.

W. Groberg This spring, Pahsimeroi steelhead being reared at Niagara Springs underwent IHN epizootics; at Hagerman National Fish Hatchery and at Pahsimeroi the same stock of fish had no losses. Harold Ramsey sent me three rainbows from the headrace at Niagara and one had IHN. These findings suggest to me that the fish in the headrace were responsible for the IHN epizootic.

B. Busch Have you seen Niagara Springs?

W. Groberg No.

B. Busch You should try to disinfect it.

W. Groberg What I am alluding to is we have to be careful about accepting both vertical and horizontal transmission if only horizontal transmission is responsible for epizootics.

D. Mulcahy Warren and I have found this out, working together. One. guy comes up with a good argument, the other one thinks of something else.

J. Rohovec Couldn't each mode of transmission be taking place?

W. Groberg We need to know the mechanism for the epizootics. If it is horizontal, we need to know that. If it is vertical, we need to know that. If it's both, we need to know that too.

B. Busch I feel, based on our experiences, that we can tell whether there is horizontal or vertical transmission, depending on when and where it occurs in our hatcheries.

W. Groberg As long as you are sure there are no carrier fish in those springs above.

B. Busch That is certainly a consideration but it has not been well supported from an historical or practical viewpoint. For instance, in a hatchery building with twenty incubators you may only have one incubator break with the virus and that lot has inveritably been associated with a suspect lot of fish at the broodstation or an uncertified group of eggs.

W. Groberg You are doing probably the best work experimentally. Will it be published so that others can take advantage of it?

B. Busch Publication of this type of data and information is not encouraged by private industry for proprietary and competitive reasons.

W. Groberg Most of what you have is positive, and would help.

B. Busch If any of you are interested, we would certainly invite and support cooperative studies as long as no proprietary, confidential, or competitive information were released in publication.

W. Groberg You have the opportunity to do things we just cannot do.

K. Amos None of those stations could afford an economic loss in any short period of time that would be offset in a long period of time. So essentially, you are not going to try to eliminate and disinfect the stations that are on continuous water supply. I should say that the hatchery owners would not be in favor of destroying their stocks and disinfecting. They can not afford it.

B. Busch If YOU would mitigate their losses both in terms of production and market as well as guarantee them the disease would not recurr, I am sure they would consider shut-down and disinfection.

L. Ray It would be comparable to you people in State and Federal agencies saying the hatchery you work at is contaminated and you are going to dry it up so fire me for the next two years. You guys would not be enthusiastic anout drying these hatcheries up either. You are not just putting that farm out of business, you are putting a thousand people directly out of jobs for a year. You cannot do that.

B. Busch Ken Wolf is known for a comment with regard to the fact that infectious disease in a hatchery is not efficient operation. In theory, I cannot argue with that. In practice, there is no substance to it. As more fish health regulations are written for Oregon, Washington, California and elsewhere, our own business is strengthened because those areas become less productive, profitable, and competitive.

W. Groberg They just become academic, even on our part.

L. Ray California has the potential to raise as many catfish as Mississippi but they will never do it as long as they have the regulations in force that they have there. They have destroyed any possibility for a real industry down there.

B. Busch The basic problem is the differences in need and priority between a resouce fishery and a food fishery. They can have two entirely different sets of goals and objectives and at times can even be at odds with one another. There is no question that IHN and other diseases are detrimental to migrant stocks. However, to the food fish industry, they are probably not a problem that cannot be managed around and in which erradication is not an economically viable alternative.

E. Wold You mentioned that ninety-seven percent of the fish are processed?

B. Busch It is a difficult figure to come up with exactly, but the amount of live fish being hauled out of the area is inconsequential.

E. Wold What happens to the eighteen million pounds of material processed away from the product that is sold, viscera and such?

B. Busch Virtually all the processing waste goes into the mink food or pet food industries at the present time.

D. Mulcahy It is nice to have the opportunity to talk. I feel there has been an estrangement between the private people and the rest of us - I am not sure why. I am sure'there are things we are doing that are of interest and use to you people. I hope we can knock down some of the barriers.

L. Ray Being raised in the catfish industry, we do not have this barrier. It is complete communication with state, and universities. You could not have a better rapport, better working relationships between industry and research as well as with Fish and Wildlife. I would really like to see an improvement in that line with trout. It is just unbelievable the cooperation and closeness with the catfish industry and Fish and Wildlife and the research community.

K. Amos How would you say closeness was between catfish producers?

L. Ray Yes, that is where it is.

K. Amos I would say there is no closeness between competitors in Southern Idaho.

L. Ray I misunderstood. You mean, what is the competition between individual producers?

K. Amos Right, as far as closeness. Part of the problem in solving the IHN problem in southern Idaho is related to the competition between companies.

L. Ray An example: We have that competition in the catfish industry producers, in the processing end of it for example. One guy has been in for a long time and everytime he has gone, he's taken all his markets with him. You can imagine the competition among the individuals. Just like in Idaho. But they have the good fortune of the industry developing around Stuttgart, with Myers, Kermit, Harry, and Mayo and those guys working with the farmer to develop mutual respect. The University, the whole program, is so close.

K. Amos Is it because the trout farmers developed their own technology? They didn't need anyone, unlike the catfish farmers?

L. Ray When Wildlife Vaccines decided to develop a vaccine for trout, they came up to the industry and asked where they could do field tests. Everyone said they would do them. Last year they went to the Catfish Farmers Convention and I introduced them to the major growers and everyone took them to Tom Spiegler and said this man would do it for you at Mississippi State. They wanted proprietary information and they knew if the University did the field tests, they would be published. It created a bit of a problem. They went to the University and said here are the people we depend on for this. The trout industry depended on themselves.

B. Busch This situation in the trout industry goes back to the days of the hepatoma scare. The Idaho industry is controlled by four privately held companies. These people have vivid memories of getting burned very badly during the hepatoma situation and hold researchers and biologists in the U.S. Fish and Wildlife Service, and the universities directly responsible. However, we are now beginning to see the industry going from a closed system to a larger, more diversified corporate structure with management being turned over to younger people. They are beginning to go out more and look for advise, encourage cooperative studies, etc. Don't write them off completely.

D. Mulcahy I never have. I found it peculiar. It happened before you and I came along.

L. Ray There will be fifty people from government agencies if you go to a catfish conference. There will be one or two at a trout conference. We are making an effort this year to draw in agency and government people. Bob is trying to get people to the convention to give papers and open communication. It is for you guys to come and get acquainted.

B. Busch The U.S. Trout Farmers Association has formed a research committee of which Dr. Bob Stevens of the U.S. Fish and Wildlife Service is a member.

L. Ray Trout Farmers Convention next year will be here in Portland. So you guys come to it, in September. .

D. Mulcahy As a private grower, given that basic studies, IHN in particular, are done with public monies and labs, what would you have us do? Or see us do?

L. Ray I do not think you can do too much research. I would like to see the entire U.S. Trout Fishery Association, through Research Commttees, come up with a list of priorities and update it every year with assistance from you people. With catfish, every year Stuttgart brings in a research program and presents it to the Catfish Farmers Research Commission. We have the communication and input on where to go. The biggest asset I would like to have, with IHN, would be to how to get a fish up to two inches, without breaking with it. Bob says they can stand ninety-five percent mortality. It hurts. The fastest way to gain the most information in the least amount of time is to survey the IHN problem, find out all the breaks that have occurred, what have done with them, and by shear information gathered, you could cut the mortality in half. Dean Frank in sheep industry cut mortality in half in one year by surveying the industry. Trout culture is at that stage. We can learn more faster by exchanging infomation than we 'can any other way, and we do not have that now.

T. Barila That is what BPA can help out with, through the mandate of the Regional Act. What makes this legislation important is that it is the first time an agency has been mandated to "do" a program, with funding.

B. Busch If you think you will get the private trout growers in Idaho to give all the necessary information freely at this point in time, I think you are wrong.

D. Mulcahy Bob says he has a problem in that it does not sound like he can get the information out, so who can get it, or can compile it?

T. Barila This is still the vehicle to at least list the priorities in the areas to be examined before a lot more time is wasted, and to try to follow a program the Council's Put together.

B. Busch I don't believe there is anyone in that position in Idaho at the present time who can give you that information accurately. I doubt if the majority of companies would offer it at all.

L. Ray What Bob says is true, but the information we can get is still the most valuable information we can get in cost and time. The problem is everyone sitting back with their pet projects, overlapping and duplicating others, no communication in between. In the catfish industry, ten to twelve years ago, they formed the Anderson committee which reviewed all research being done on catfish and found that eighty percent of the money was being wasted. It really shaped up the research committee.

B. Busch One basic difference, however, is that research on catfish is done only as a cultured food fish whereas with trout we are dealing with wild stock management, mitigation, etc. as well.

D. Mulcahy Yes, I suspect we will have two different sets of priorities.

B. Busch The private trout industry sits back and looks at all the millions of dollars being spent on trout research at the present time and consider it as a waste of money because it does not follow their priorities.

L. Ray Stuttgart was established with a mandate for the commerical industry and not sport fishery.

D. Mulcahy That is the only Fish and Wildlife Service lab set up that way and it has worked well.

J. Rohovec The commercial salmon industry communicates well with state and Federal agencies and universities. I do not know what the difference is.

B. Busch It is a much younger industry and in terms of culture, pen rearing or ocean ranching is not yet a profitable industry. It also does not have the history of regulatory agency association and involvement, hepatomas scares, etc., that go along with the trout industry.

L. Ray Another thing that encourages you people to look at the commercial industry-for several years aquaculture was the hottest item in Washington. The second largest trade in the U.S. is fish products. Our deficit/balance of payment

as far as export/import is oil and fisheries. This is not an item that is going to die, because Congress is going to continue to resurface with research money to develop an aquaculture industry in the U.S. This would be the best source of money for the fish and wildlife research community to get your hands on. Washington pumped a lot of money into sea grants and there really has not been a viable aquaculture item developed from all that research. We've had a catfish and crayfish industry in Louisiana that is probably larger than the U.S. trout industry today. Crayfish is coming on strong. It is a growing industry. The catfish industry is in bad shape. They have one hundred million pounds to process, but its still the most profitable crop in Mississippi. Crayfish is showing the fastest growth and profit of any agricultural crop in Louisiana. It is expanding into Mississippi and Texas. Aquaculture is coming and its because of this deficit/balance of payment. The government does not like all that money going overseas.

K. Amos Is there any financial support provided by Catfish Farmers to help with research?

L. Ray No. Just the other way. We are just seeing it change. The feed manufacturers are contributing three dollars per ton now at the request of farmers. Most is going for market research. Also for matching funds for government research and production. The industry is coming up with a quarter of a million dollars this year. The catfish industry has stayed on top of the legislature, or above where the trout industry has. This has been the university and people like Stuttgart that stay on top and inform us of what is going on. It is a good example of the type of coordination and how it can work.

W. Groberg Maybe the time is right, given the IHN problem, and that money is available for the Columbia Basin fisheries resource and that both segments share a common problem.

B. Busch Yes, I think there is fertile ground to be cultivated in this area of renewed cooperation and a common cause.

L. Ray When you go over there, you may get run off a hatchery or two, but don't let that discourage you. Keep the couuuunication going. There are a lot of younger degree people, who are assuming roles of responsibility. These will be the communication lines in the future..

B. Busch I would think that the average age of a commercial trout hatchery manager in the Hagerman Valley is now thirty to thirty-five years old and many have college or graduate degrees.

L. Ray — Ten years ago, there were one or two degree biologists in the entire valley. There are a lot of degree people there now.

J. Leong — Do you guys ever meet together?

L. Ray — No formal organization. No Idaho chapter of Trout farmers. When something happens -

B. Busch — It is like a close family, we fight like Crazy among ourselves, but when something happens to any one of us we can pull together fast. IHN is common ground that can bring us together. BKD has the same effect.

W. Groberg — And if we do show them that something would work, they would probably listen?

8. Busch — Yes, but it has got to be practical and cost effective. They are business men as well as fish farmers. They look at everything in terms of its economics.

H. Ramsey — A couple of years ago, Leo Ray and Don Campbell were responsible for establishing Idaho Fish Culturists Association and we did have some meetings in the Valley to discuss all aspects of problems. I do not know what happened. I guess we did not get notices out on time and it rather disintegrated. But we did have several productive meetings.

L. Ray — With the young biologists that are there now, they would really like to have the exchange of information. There is some problem among some of the companies about wanting to exchange information. Some say you can go and listen but do not talk. But that is not a major problem.

W. Groberg — Are most of them members of the Trout Growers?

L. Ray — The farms **over** there, yes, most are members of the U.S. Association.

W. Groberg — Could the Trout Growers be used as a medium?

L. Ray — The U.S. Trout Growers Association? No, there is a problem there. Three of the four major farms belong to the National Association and support it strongly. They do not try to dominate it. In the past, they have tried to dominate it and the U.S. Trout Farmers Association almost died. The people in Idaho respect the USTFA and they feel a real need for it. It would be easy for Clear Springs to say we have half the nation's production and we do not need the U.S. Trout Farmers Association, but when it comes time to vote they only have one vote instead of several hundred that the Association would have. So Clear Springs supports the Association very strongly but does not try to dominate

it. There is another segment of that industry over there that has been overlooked and really I am more a part of that segment of it than I am of this part I have talked about. And there are probably two or three hundred people with farm ponds that grow from a million pounds of fish down to ten to twelve thousand pounds. And this is a segment of the industry that represents eight to ten million pounds of fish that are not involved in the industry. It has been a farm pond situation that the four major producers supplied the fish and feed and paid them on the gain. In the last two to three years, the major producers had more fish of their own than they wanted, so they did not stock any of these. These facilities are mostly empty now. A lot of these people are getting interested now in growing their own fish. I simply started growing my own fish and processing them. Because I have market contacts through the catfish, I have been able to stay alive. Like I told Percy Green one time, I can stay alive if I have a pound of catfish to sell for every pound of trout I sell - or that I give away. This other segment is one that has no background, that needs education. I think most of them are going to be raising fish. They may get more involved and I think they would work very closely with the universities.

D. Mulcahy One reason preventing our greater communication with Idaho growers is that the estrangement has been there for so long we do not know how to get to you people.

B. Busch But it was there before you and I came on the scene. It is up to us now to resolve these differences and work to develop a more cooperative air.

L. Ray There is not a week that goes by that I don't talk to someone at Stuttgart, or Mississippi State or Auburn or Oklahoma or Texas A&M. We have had at least one or two students up here every year for the last four of five years from that industry down there. I know very few people in the trout industry in my back door-as far as government and university are concerned. And I have survived by my communications with my research community in the South. They are very important.

W. Groberg Perhaps if we persevere, we will have something concrete for the Trout Growers Convention next September and we can open communication channels by then.

L. Ray The college students go the Catfish Farmers Convention every year to get acquainted with future employers. And the farmers go there to meet these students with prospects of hiring. If I need to hire someone in catfish, I call the university.

J. Leong That raises a lot of interesting points which I think Teri would like to cover.

T. Barila Yes, I'll briefly touch on it tonight, and the main part tomorrow. One of the main reasons BPA was willing to support this workshop was to try to get some kind of a product, besides exchanging information from you all that we could use in trying to formulate our responses to the Draft Program that has been issued by the Power Planning Council. We would like to give them something of merit and weight to show them that there is a lot of concern about how this whole program is going to work. The Council has identified a need for a systemized approach, and BPA supports that, but we do not see that approach in the Draft Program. We see a lot of recommendations for work that will hopefully pay off, but BPA is hesitant to jump into funding many single activities that do not fit into some overall program. And we felt this workshop approach-specifically, IHN, might be a valuable example to give the Council and say look, here is one workshop on a big problem and this is what we came up with or at least to begin some kind of approach that is workable. If one example, like the IHN workshop, can be given to the Council, it may help to structure a better approach than what they have given us in the Draft Program. BPA is committed to following the program, while still retaining our authorities in dealing with ratepayers' monies. If we don't see a program, it will be difficult to follow something that does not exist. Disease is far enough away from BPA's main focus that it is hard to know how much involvement in disease work there will be. BPA is concerned primarily with direct river operation problems. If we could have some type of program, or outline, to submit to the Council, it would be helpful.

L. Ray One and a half years ago, the industry got together and said the number one problem was strawberry disease. I do not think there is any doubt that they would probably say IHN was number one today. Stawberry hits a smaller number of fish but they are market size fish and cost you a lot of money. IHN hits a smaller size fish, but scares me more.

T. Barila Of course this is just one aspect of the entire picture that BPA in concerned with.

W. Groberg What you are asking for is an outline for research needs?

T. Barila Or a prioritization, or approach that this group would see as a viable attempt in getting started with IHN.

W. Groberg I have an outline of what I see as needs, and maybe with what we could put together here, it would serve that purpose.

E. Wold Fishery agencies, through the Artificial Production Committee of the Columbia River Fisheries Council, are putting together a step-down plan for research needs of all fish diseases.

T. Barila That is the ultimate goal.

E. Wold This workshop you want specifically for IHN.

T. Barila BPA is relying on the fishery agencies for input, but even this two day workshop would be effective for BPA's involvement if presented to Curt Marshall. It might be the best comment we could make in our response on disease work to the Power Planning Council. And the disease work is just one component of the Artificial Production objectives, the others being Adult Migrant Survival, Juvenile Migrant Survival, Natural Production, Wildlife and Resident Fish.

K. Amos Who makes the final decision on the prioritizaion of use of resources, and when does that decision have to be made?

T. Barila Comment period ends October 25. The final Program will be released November 15.

K. Amos But as to how the money will be spent and on what?

E. Wold The workshop approach is a good idea, but if you are going to call one for each disease, you will have the same people around the table each time. We might as well get all the priorities established, and that is what we are working on.

T. Barila BPA has not been involved to know what is going on, and that is what were are trying to do now.

K. Amos The Idaho hatcheries' broodstocks are disease-free?

B. Busch For the most part yes, with some exceptions.

3/ D. Mulcahy When you say you're checking the adults year after year, you've got a great opportunity to answer questions we may be facing with steelhead, who are also repeat spawners. I wonder if the level of virus produced by the fish is constant year after year. Will a fish that produces 10^6 pfu per ml produce that amount next year?

B. Busch Our experience with the one stock that was infected and have since spawned twice, as two and three years olds, we have not seen any indications that there is a decrease in virus titer, but our data to date is insufficient to draw any conclusions. We are also looking at the heretibility of resistance to IHN virus. We have a population of IHN virus epizootic survivors that we're following. We go through those fish every thirty days and examine all the tissues for virus. We plan to carry these fish through at least two spawnipgs. They're just now coming into their first year of maturity and won't spawn till November, 1983.

E. Wold What is the number of fish in the brood stock that you're carrying year after year?

B. Busch At 58°F, we have five thousand yearlings. At the brood stock facility, we have many thousands of fish potentially exposed to the virus. We are checking this stock for IHN as they spawn.

G. Taylor You're taking a ninety-five percent loss?

B. Busch When there is demonstrated vertical transmission through the egg, yes, we can take up to ninety-five percent loss in the sac fry in the incubators. In our throw-out system at our broodstock facility, we've had only one breakthrough of virus in'ten months so we've virtually eliminated, at least functionally speaking, vertical transmission. The majority of our losses now occur in post-ponding fry due to horizontal transmission within the hatchery and average about thirty percent.

4/ D. Mulcahy Do you feel disinfecting total water supplies is a problem?

B. Busch At this point in time, for most hatcheries,. it is not necessary. Leo is an exception as he's drawing his water out of a ditch that's rather long. Our major water supplies appear to be free of IHN. We do have some escapement into our water supplies and are taking measures to minimize it. We also have a problem in that some of our supplies are only partially diverted and the State's saying they want wild stocks to be able to migrate up into them to spawn.

K. Amos Box Canyon, for example?

B. Busch Yes, that's a good example.

W. Groberg Given that there are fish above, in some of those spring water supplies, can you really be sure this peracute form is a result of vertical transmission?

B. Busch That is a good point, and the only answer I have is that to the best of my knowledge we have never had an outbreak of IHN virus in a hatchery building in a certified disease-free stock. I am convinced that what we see in our hatchery buildings is true vertical transmission and we're not getting horizonal transmission from the water supply in most cases.

We do control virus very well in our hatchery buildings where we can properly disinfect by shutting off the water, drying ponds and disinfecting. When we do have a break in a hatchery building, through vertical transmission, we can disinfect and prevent its spread or recurrence. Once the

fish are ponded outside, however, there is no way to properly disinfect for several reasons, one of which is the design of our facilities. In the newer block designs, the water comes in to the hatchery in a common head ditch, goes through several series of raceways, recombines in another common head ditch, and goes through more raceways, before being recombined again. There is no way to disinfect a single pond or series. There is no way to divert water out of a pond, to stop the flow, or dry it up. When you start talking completely dewatering hatcheries and shutting them down, to properly disinfect them, keep in mind that these facilities are in production twelve months of the year. We had a situation, unfortunately, where we lost part of a water supply in a large production hatchery. We had no choice at the time but to shut it down. We used that opportunity to maximum advantage to disinfect it. So far it has been quite effective. However, if you were to take one of the large production hatcheries in Idaho, say Box Canyon with almost a seven million pound annual production capacity and consider that it would take you about eight months just to cycle the fish out of there before you could shut off the water, dry it out, and clean it up, and the only way to restock it would be with certified disease-free eggs which require another twelve months to put it back into full production, that is a twenty month closure on a seven million pound production hatchery. Add up the endenmification costs at $1.85/pound plus lost markets and . . . virus does not cost us that much.

D. Mulcahy You can live with the IHN mortality?

B. Busch It comes down to simple economics. We can live with the IHN virus mortality at its present rate.

D. Mulcahy How much higher would your percent mortality have to go before you couldn't live with it? Could you live with ninety percent?

B. Busch I hate to say it, but I think we almost could if it occurred in small fry fish. Right now, we average thirty percent acute mortality in fish approximately four grams in size, average weight. Our secondary loss to the scoliosis problem in larger market fish is economically more important than the thirty percent mortality of the fry fish.

D. Mulcahy But it is still IHN.

B. Busch It is still IHN.

G. Taylor What is the total, overall losses?

B. Busch To what?

G. Taylor IHN and scoliosis problem.

B. Busch Thirty five percent - thirty percent mortality and four to five percent scoliosis.

L. Ray You buy four eggs to get one to market.

D. Mulcahy How many of those three that don't, do you attribute to IHN?

B. Busch One of them. I would estimate that we'll hatch almost eighty million eggs this year in the Valley and produce somewhere between twenty-eight and thirty million pounds of trout.

E. Wold Approximately how many of those, percent wise, come from outside the State?

B. Busch About sixty percent.

5/ J. Rohovec You indicated that if you have an IHN epizootic early in the game, you do not see the disease later. Wouldn't you hypothesize from that that you have some kind of protective mechanism? Immunity does not have to occur in serum, especially in the case of virus.

B. Busch There's no question that there is some kind of protective mechanism developed. I do not think it is the classical humoral immune protective response we are used to dealing with in terms of IPN. I think we are looking at something entirely different.

D. Mulcahy Perhaps you are culling out the most susceptible in the earlier epizootics, so less virus is produced later to induce an epizootic.

B. Busch When we have a viral outbreak in a top fry pond, the fish right below them most likely have gone through a similar epizootic six to eight weeks earlier and are getting the full brunt of virus in the water coming down. We have never seen any reoccurrence but your point remains a possibility.

6/ J. Rohovec The nature of the chemotherapeutic, does it eliminate the virus from the fish?

B. Busch No, the virus is still present. We can demonstrate that. We can treat for seven days, take the fish off treatment, and they will break with the virus. The same thing occurs after fourteen, twenty-one, or forty-five days of treatment.

D. Mulcahy Why not keep them on?

B. Busch The treatment is no longer cost-effective after the fish attain a twenty gram size.

L. Ray When you raise them on this, and get them up to three or four inch size, do you reduce your mortality?

B. Busch It does not appear to. We have had two trials that went to forty-five days. In both trials, when treatment was stopped after forty-five days, mortality was within five percent of the control group mortality forty-five days previous to that. We were hoping to see a reduced mortality but that was not the case.

L. Ray Those hatcheries that have IHN virus in their water supply could possibly use the drugs till they get their fish to size.

K. Amos Sounds like they have to go through an epizootic to get the proper number of organisms to derive this protection.

B. Busch That is the whole point I am trying to make. We need to work on the pathogenesia of this disease to find out what form the protective basis is against recurrent and supra infection. There is a definite need for a better understanding in terms of the potential for coming up with some type of live, modified or killed vaccine.

Current Fish Disease Control Policies Affecting the Columbia River Basin [1]

John S. Rohovec

Department of Microbiology

Oregon State University

Corvallis, Oregon 97331

1 Oregon Agricultural Experiment Station Technical Paper No. 6568

The Columbia River Basin extends into and includes areas of seven states of the United States (Washington, Oregon, Idaho, Wyoming, Montana, Utah and Nevada) and two provinces of Canada (Alberta and British Columbia). Within the Columbia Basin, the rearing of fish, especially salmonids, is conducted by private individuals, commercial enterprises and by state and federal agencies. Although diseases can be a limiting factor in the propagation of those animals, there is presently no comprehensive fish disease control policy which has been adopted and is in effect in this area. With the wide range in geography and in individual interests it would seem that such a policy would be difficult to formulate.

However, precedents have been established to the contrary. In a rudimentary form, the United States government initiated fish disease control legislation in 1958. The Title 50 law was amended to regulate the importation of fish or fish products which are infected with either the agent causing viral hemorrhagic septicemia (VHS) or Myxosoma cerebralis, the etiological agent of whirling disease. This legislation was enacted after whirling disease was introduced into the United States and had been responsible for devastating epizootics among trout populations in the eastern part of the country. It was realized that VHS, a disease .of salmonids in Europe which is not found in the U.S., should also be included in the Title 50 regulations. As a result of this law, M. cerebralis has not become geographically widespread and is contained, for the most part, in the eastern U.S. Thus far VHS has not been introduced into this country.

Title 50 involves the entire nation but is limited in &cope since it concerns regulation and control of only two specific fish pathogens. However, there are fish disease control policies and regulations which have been enacted by state governments which are more stringent than those of the

federal government (Fryer et al. 1979). Of greater importance to this discussion is that there are also fish disease control policies which involve two distinct regions in the United States. These are the Colorado River Fish Disease Control Program and the recommendations of the Great Lakes Fish Disease Control Committee. These regional policies have factors in common but also contain features which are unique.

The seven states of Arizona, California, Colorado, Nevada, New Mexico, Utah and Wyoming which manage waters of the Colorado River drainage agreed to a fish disease control policy in 1973. The responsible agency in each of these states is mandated to make every reasonable effort to prevent the introduction of the following fish diseases into the Colorado River drainage system:

1. IHN- Infectious Hematopoietic Necrosis of salmonids
2. VHS- Viral Hemorrhagic Septicemia of salmonids
3. ccv - Channel Catfish Virus Disease
4. Whirling Disease - Myxosoma cerebralis
5. Ceratomyxosis - Ceratomyxa Shasta
6. Bacterial Kidney Disease - Renibacterium salmoninarum
7. European Gill Rot - Branchiomyces sp.
8. Blood Fluke of Salmonids - Sanguinicola sp.

A fish disease subcommittee evaluates the findings which are provided by a certifying team of fish disease specialists from the participating states and the federal government and maintains records of disease incidence in hatchery histories.

The fish disease control policy of the Colorado River drainage has the following provisions.

1. Before any fish cultural station may stock game fish or conduct fish cultural activities in the drainage system, the station must be certified free of the pathogens listed in the policy.
2. When fish cultural stations experience significant fish losses and have fish showing clinical symptoms of any disease, it cannot plant fish into the drainage system until the disease problems are solved and the station reinspected.
3. All game fish that federal, state and private fish cultural facilities plant into the Colorado River drainage system shall be free of the diseases or the pathogen6 inducing the diseases listed in the policy.
4. Any certification is immediately void upon confirmation that any of the listed diseases are established in the certified hatchery.

The policy is a result of a resolution adopted by the member governments and indicates, in part, that diseases of fish have become widespread, are critical problem6 and that their dissemination is a matter of record. The resolution also states that information concerning the impact of siseases on indigenous fish is insufficient and that introduction of diseases not endemic to the Colorado River drainage can be prevented through adequate inspection and restriction of imports. To contend with diseases already present in the drainage, the resolution states that it is technically possible to restrict or eliminate pathogens by way of dilution and this can be achieved through a concerted effort to avoid further additions of pathogens and distribution of known disease carriers.

The Great Lakes Fish Disease Control Committee which consists of individuals representing administrators and fish pathologist6 from eight states, one Canadian province, the federal governments of the United States and Canada, and representatives from commercial fish culture groups have the objective of protecting and improving fish health in the Great Lakes basin. The committee has the following policy statement (Sippel, 1982):

"To work toward the attainment of fish disease control in the Great Lakes basin, it shall be the policy of the Great Lake6 Fishery Commission to encourage each member agency to

- Develop, by 1980, legislative authority and regulations to allow control and possible eradication of fish diseases;
- Prevent the release of seriously diseased fish;
- Discourage the rearing of diseased fish;
- Prevent the importation into the Great Lakes basin of fish infected with certain certifiable diseases;
- Prevent the transfer within the Great Lakes basin of fish infected with certifiable diseases; and
- Eradicate fish diseases wherever practicable."

The certifiable diseases include two emergency diseases, whirling disease and viral hemorrhagic septicemia, and ceratomyxosis, infectious hematopoietic necrosis, infectious pancreatic necrosis and enteric redmouth. In addition, bacterial kidney disease and furunculosis are monitored.

The Great Lakes Fish Disease Control Committee has defined a recommended model to be used in developing fish disease control programs. This document was formulated using the Colorado River drainage disease control policy and elements of other similar document6 as guidance. The model contains procedure6 for inspection and diagnosis of disease agents, a hatchery disease

classification program, lists of diseases to be regulated, disposition of diseased fish and other specifics of importance to a disease control program.

A unique aspect of the fish disease control program of the Great Lakes basin is that it depends on voluntary agreements and peer pressure instead of formal regulations. The recommendations advanced by the Committee are provided to aid member agencies in the continuing development of fish disease control programs to assure they serve the best interests of all Great Lakes fishery resources. The Committee states "that it is in no way seeking fish disease control authority".

There have been no attempts to formulate a comprehensive fish disease control policy for the Columbia River basin; however, most individual state and federal agencies operating in this region have fish disease control guidelines which they follow. Idaho is the only state in the Columbia River drainage which does not adhere to any formal fish disease control guidelines other than that provided by the Federal Title 50. Each of the remaining states, the U. S. Fish and Wildlife Service (USFWS) which represents the United States government, and the federal government of Canada have some form of fish disease control policy. This discussion will be limited to the policies of Washington, Oregon, Canada and the USFWS whose guideline6 are summarized (Table I).

Each of these agencies include a list of specific diseases which are part of their control policies. These are usually categorized according to the severity of the disease, the degree of its distribution and the difficulty of its control. In all instances viral hemorrhagic septicemia (VHS) is considered an emergency disease because it is devastating to populations of fish, it has never *been* detected *in* North America and, because it is caused by a virus, it is untreatable. Whirling disease, caused by Myxosoma cerebralis,

is also considered an emergency disease by all agencies except. the USFWS. This disease also causes high mortality and no chemotherapy is available. The disease has been introduced into North America, albeit not into the Columbia River basin.

Each organization has diseases which are classified as certifiable and include these emergency diseases. In each instance, these include the viral diseases, infectious pancreatic necrosis (IPN) and infectious hematopoietic necrosis (IHN). Channel catfish virus (CCV) and Herpesvirus salmonis are viruses included by some agencies. It is noteworthy that Canada's guidelines include all filterable agents which replicate and cause cytopathic effects in cell lines of fish. Canada, the USFWS and Oregon include the bacterial agents, Aeromonas salmonicida, Yersinia ruckeri, and Renibacterium salmoninarum, and the protozoan, Ceratomyxa Shasta, among their certifiable diseases.

Each organization provides that diagnostic methods to be used for the detection of the certifiable diseases are described in either the "Fish Health Protection Regulations Manual of Compliance" (Department of Fisheries and Environment 1976) or *'Procedures for the Detection and Identification of Certain Fish Pathogens" (American Fisheries Society Fish Health Section 1979). Inspections for certifiable fish pathogens are required in most instances, at least one time per year and usually correspond to the time at which fish are spawned. There are special requirements for the detection of M. cerebralis and, of course, examinations are conducted when fish are experiencing increased mortality or epizootics.

Pathologists from each agency are responsible for managing the health of fish reared by that agency. Each organization also maintains and provides lists of individuals who they deem acceptable to perform disease

certifications or inspections to comply with that organization's disease policy. The State of Washington has determined that by 1984 only those individuals who are recognized by the American Fisheries Society Fish Health Section as Fish Health Inspectors or Fish Pathologists will be qualified to perform fish health certifications for Washington state.

Perhaps one of the most important aspects of any disease control policy is the development and maintenance of disease histories of hatcheries and watersheds. These histories are records of all diseases and other pertinent information, such as fish transfers, which have occurred at a particular site. Each agency discussed has some method or provision for keeping disease histories of hatcheries under their jurisdiction.

Disease histories are especially valuable in making decisions concerning transportation of fish or fish eggs from one site to another. Although not stated precisely, the issue of transport permits is one of the major apsects of Oregon's disease control policy and decisions are often based on histories rather than on individual inspections. The USFWS classifies their hatcheries according to diseases which have been detected in fish at each location and do not allow transport of fish from one installation which would downgrade the disease classification of the receiving site.

The major objective for implementation of fish disease policies by each of the agencies discussed is to protect fish resources by preventing importation and dissemination of fish pathogens. The documents of all agencies, excluding Canada, also have some provisions for the disposition of diseases when found *in* populations of fish. In each instance, if any emergency disease (whirling disease and VHS in Oregon and Washington, VHS in the USFWS) is detected, the eradication of the disease by destruction of the affected population of fish is mandatory. This is followed by disinfection of

the facility, cessation of egg and fish transports, and attempts to contain the disease and sanitize the watershed. Procedures for dealing with certifiable diseases, other than those emergency diseases, are less defined. Usually an effort is made to control the disease by a quarantine of fish at the hatchery, preventing the spread of the disease by isolating it in the specific watershed or by destruction of the affected stock of fish. In some instances there may be no effort to control certifiable disease which have bacterial etiology. The state of Washington has recognized in its disease policy that IHN and IPN, which are both certifiable diseases, exist in the Columbia River system. Anadromous salmonids from the Columbia River and progeny of those salmonids are not permitted to be transported to watersheds which do not flow into the Columbia River, but fish which have been exposed to the maintstream of the river can be moved within the Columbia drainage provided they have not experienced clinical IHN or IPN or are not progeny of fish shown to be carriers of IHN or IPN.

After recent isolations and detection of IHNV with resulting epizootics of IHN in the Columbia River basin, those whose fish are affected have followed several different avenues. These methods include: no attempt to control the disease and the rearing of survivors of epizootics; an attempt to rear eggs from virus-free adults; and total destruction of affected populations and sanitizing hatchery facilities. Some agencies practice more than one of these techniques. This example indicates the difficulty which will exist in the formulation of a comprehensive disease control policy for the Columbia River basin. However, such policies have been designed for other regions and using their guidelines and those of individual agencies *in* the Columbia basin it should be possible to design a program for controlling diseases of the valuable fishery resource of this region.

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Table I. Summary of fish disease control policies of selected agencies in the Columbia River basin.

	Canada	us FwS	Oregon	Washington
Certifiable Agents	AS,RS,YR,MC,CS, any filterable agent causing CPE in fish cells, VHSV,IPNV,IHNV	AS,RS,YR,MC,VHSV, IPNV,IHNV,CCV (CS,HS)	AS,RS,YR;MC,CS, VHSV,IHNV, IPNV	VHSV,MC,CCV,IHNV IPNV
Diagnostic Methods	Canadian "Blue Book"	Fish Health Section "Blue Book"	Fish Health Section "Blue Book"	Fish Health Section "Blue Book"
Frequency of Examinations	2X/yr	1X/yr	2X/yr	Not specified
Disease Inspectors	Agency supplies list of approved individuals	Agency supplies list of approved individuals	Agency supplies list of approved individuals	Agency supplies list of approved
Disease History	Histories kept in National Registry of Fish Diseases	Hatcheries are classified according to presence of disease	Histories are recorded with no provision in disease policy	Hatchery and watershed disease histories are recorded
Transport Permits	Required	Disease Statement Required	Required	Required
Provision for Disposition	Not specified in control policy	Eradication of VHS undefined for others	Eradication of VHS, MC; undefined for others	Eradication of VHS, MC; undefined for others

Abbreuiations: AS, Aeromonas salmonicida; RS, Renibacterium salmoninarum; YR, Yersinia ruckeri; MC, Myxosoma ccrebralis; CS, Ceratomyxa Shasta; VHSV, Viral hemorrhagic septicemia virus; IPNV, Infectious pancreatic necrosis virus; IHNV, Infectious hematopoietic necrosis virus; HS, Herpesvirus salmonis; CCV, channel catfish virus.

Questions and Answers Following J. Rohovec's Presentation

J. Leong How do you get on the list of persons who can certify eggs?

J. Rohovec It depends on the organization. We met with individuals from state agencies and OSU and compiled a list of those whom we felt were qualified.

D. Mulcahy The USFWS recognizes the state's designated list.

J. Rohovec In Washington's policy, they have stipulated that by January 1, 1984, qualified individuals will be certified fish inspectors by the American Fishery Society Fish Health Section, which will be difficult, as no one in this room would qualify for either fish inspector or fish pathologist.

G. Taylor A central file is kept in Washington, D.C. of all USFWS classifications, and histories, going back quite a few years.

W. Groberg Is that computerized?

G. Taylor I do not believe it is computerized.

J. Rohovec That is the problem with Oregon, they are filed but not really available. Kevin was helpful to me in sending Washington's policy before it was even approved. A lot of thought went into that. They provide a clause concerning the virus problem in the Columbia River Basin. They included that any fish exposed in the mainstem Columbia has the potential of being infected by IPN and/or IHN. They stipulate in their policy that those fish will not be transported to areas outside of the Columbia drainage no matter whether they have found the virus or not. If they are undergoing any epizootic or come from parents shown to be virus infected, then they do not move out of their particular watershed

K. Amos It has been an unwritten rule in Washington Department of Fisheries not to move fish out of the Columbia River.

W. Groberg In your department, not ours.

J. Rohovec That is an unwritten policy with Oregon, no Columbia River fish go to coastal streams and vice versa.

W. Groberg This policy situation has been on our minds for two to three years and we are now in the process of coming up with another draft policy. There will be some minor changes but they basically do not vary from what John has indicated here on the paper. For the viruses, if there is a new find, in a new location, we would probably still treat that pretty drastically. If it is a new find in a drainage

where it already exists, then I think we mentioned the cooperation with the state agencies involved, and what their preference is about where these fish would end up. When John mentioned the business about the inspector and a list, we tried to come up with some type of selecting system for this Title 50 domestic list. It was even recommended that the same procedure used for the Fish Health Section be used as a selecting criteria for the domestic list of the Title 50. Our program managers in Washington did not really think this was appropriate, so what we did was embark on similar types of guidelines. We have a small committee that puts together information when someone requests to get on Title 50. If they submit all the requests and the committee determines that person is qualified to get on the list, then that person is accepted. If they do not have the qualifications, or if their expertise is not necessarily in virology, then we have means to not accept those people. We are trying to come up with a list of qualified people.

E. Wold In selecting your inspectors then, you have criteria established that you look at all the qualifications or do you do it without any established guidelines?

J. Rohovec Some inspectors are selected without meeting established criteria. If you know that someone has been to certain schools, in the field, with certain training, and lab work and background-so some of it is used. But the criteria we are asking for is transcripts and letters of recommendation and that type. It is all a request though. If they do not desire to send that to us, and they want to get on the list and the qualification is there, I am sure they could get on the list. It is nothing that we can refuse.

J. Leong I am not in the business of certifying or doing any diagnostic work. For a clinical lab, in a human virus situation, the hospital pathologist has a book that they have to fill out because they have to certify whether a disease agent is present in a human. It has implications for epidemics in humans. It seems not unreasonable that something similar would be used in certifying inspectors in fish disease. There should be some standard to determine whether eggs are diseased. You want to make sure your eggs are disease-free.

J. Rohovec There is a standard that is used for the techniques, and that is either one of the Blue Books. The standards for the individual doing the test are much more nebulous. In this industry there is not a real problem because the people in certification are well known enough that their reputation qualifies them.

B. Busch There is such a wide variety in the training and education of these people. It is not like human medicine where there are established schools and education. We have people that are more than qualified to do this work, and some of them may not even have *a* college education, certainly nothing more than a bachelor's One may be trained in microbiology, another in fisheries. Much is based on experience, not education. It is difficult to come up with a set of criteria that fits this wide diversity of qualified people.

J. Leong The question was not the qualifications of the individual, but the way the tests were run.

B. Busch That is pretty well stand-ardized.

J. Leong Who checks them?

J. Rohovec Nobody checks to make sure you are using the Blue Book methodology. Something I have read that is interesting in the compilation of these policies, is that it says you can use Blue Book techniques or better. And sometimes that "or better" is the interpretation of the inspector, and one inspector may interpret it to be the quickest, and another interpretation'may be to do something more stringent.

B. Busch I will tell you something that is missing that clinical, human medicine has, and that is some system of check samples. That is one of the biggest problems we have, and I think everyone has. In many instances, you are dealing with things you do not see on a routine basis. To have some series of check samples to go through these labs with, even on a voluntary basis, I am sure everyone would be interested in doing it periodically.

D. Mulcahy Were you at the Springfield meeting a few years back?

B. Busch No.

D. Mulcahy I proposed to send samples out on a voluntary basis, to take the time to prepare virus samples. The samples would be distributed in a blind experiment. I even arranged a way so I would not know how a lab did in the test. They would be the only ones who knew how they did, so it would not appear to be a regulatory move. It would be a self-check. With two or three exceptions, the reaction was one of fear. They did not want to know.

B. Busch I can understand that. It is not you, Dan, it is where you are. If it could be done through the Fish Health Section, it would be better received.

D. Mulcahy You mean because I work for the Federal government?

B. Busch Yes.

W. Groberg That could be the next step. The Blue Book was written, there has been an attempt to certify two types of professionals. The next thing down the line should maybe be a systems check.

B. Busch I think too that it would have to be voluntary, to begin with. And I think if you did it on a voluntary basis you would find that the type of people who had reservations with regard to it would start utilizing it.

L. Ray This shows how ridiculous some of those regulations are that you impose on us. You give people the authority of life and death over us, as far as private enterprise in the industry. If somebody were to get a grudge against an operator out here, he could simply say he has IHN in his egg operation.

D. Mulcahy It is not that simple to get on that list.

B. Busch The point John was making was that the group is small enough to where everybody knows everybody and if there is any questions of somebody writing a certification that somebody else does not know, it is followed up on.

J. Rohovec It is even policed in some nebulous manner too. If several certifications come from an individual and the same samples are run in another lab, and if the results continue to be diverse, that person might be eliminated from the list.

L. Ray People at California Fish and Game there would sure argue with that. They would not accept Rangen Research results.

S. Roberts They would not accept anyone but their own state, Idaho?

L. Ray Because of things that have happened.

D. Mulcahy That is a key statement, "Because of things that have happened" because it is an unstructured system at the moment. You are also opposing the introduction of regulations. I hear you saying two different things.

L. Ray If a regulation is going to be imposed, there should be a very definite need for that regulation. Do not impose regulations simply to build paperwork. Government does that. The channel catfish problem in California is an

example. I know a farm in California that's had channel catfish virus on it, for ten years. And everybody in the industry down there knows that every farm in California has it. But it has been illegal and all of this. It is just that new guys started to farm down there and sent in samples and they found it and got a big stir. The channel catfish virus restriction was imposed to keep Mississippi fish out of California That is the story behind it all.

B. Busch — That is the same story on the trout diseases to a large extent, to keep Idaho fish out of California.

L. Ray — California fingerlings are sold from three to five cents per inch. You can buy fingerlings in Mississippi for half a cent per inch, for twelve years now. I have benefited from this.

D. Mulcahy — Since we do not have any representation from California to defend themselves, I think we are getting one side of the view. I do not think they would agree with you. I am not saying who is right or wrong.

L. Ray — There is some of them who will-who have.

B. Busch — That is the whole point, Dan. When you start talking regulations today, you had better be sure that they are defensible.

D. Mulcahy — I bet you, if you want to start talking state regulations, or requirements for certifiers, you will have certifiers object to regulations. Nobody likes regulations. But can we deny that some regulation is needed?

L. Ray — Let me ask the next question about the laws that Oregon has on some of these diseases. Suppose a private hatchery comes down with one of those diseases, what is the procedure?

J. Rohovec — That is one of the things that---if it is VHS or myxosoma, it is written out: they eliminate their fish. If it is IHN or IPN, then it gets real shaky. Probably what would happen is there would be a big committee of fishery administrators, pathologists, people from the private company, would all sit down and try to come to some equitable solution.

L. Ray — There is some of this going on in California right now.

K. Amos — Let us say VHS showed up in Clear Springs at Box Canyon, for example-where you do not know how it got there, what kind of solution would we have? Knowing what you know about VHS, that it's not in the Columbia River drainage, trying to make a correlation to IHN, what would happen?

L. Bay It happened over there with PKD last year, and again this year. This is the problem regulations pose. Too many times regulations are passed without thought to the final solution.

W. Groberg Most of us here have been involved writing these regulations, and I do not think there is one of us here who is interested in needless regulations. Most of us are hands-on working pathologists and the last thing we are interested in is more paperwork. These regulations pose real constraints on our agencies too and we don't like them at times. But they have been written with the needs of the resource in mind. They are seemingly weak in some cases to provide flexibility.

B. Busch Are we getting into some of the areas you will be discussing, Einar?

E. Wold Oh I guess, so I will just close by saying amen.

B. Busch I have got a couple of points I want to raise but it may cover a lot of your area there.

E. Wold I think both the discussion that John had, and what I will say, should go hand in hand, and then discuss the total and obvious questions.

W. Brunson Our policies are initially designed to protect our own selves first, not to put the burden of guilt on anyone else, but to protect ourselves. And I feel they are designed with flexibility. We have left clauses in for a case-by-case basis. So if a private farmer has a legal argument, there is room for him to argue with us. It is not designed to put anybody out of business,. including ourselves, but to protect our fish. We have got to have these regulations as much as we dislike them. There is always room for improvement, and I think we have left room for that improvement in our policy.

L. Bay You really put some constraints on private industry when they get too tight. How many private farms could stand to eliminate all their inventory and start all over again? How many people would take that gamble?

D. Mulcahy Look at the Idaho trout industry. Where is the over burden of regulation there?

L. Ray That is not in Washington, Oregon or California. We do not have the regulations you have in Oregon. There is no state agency that can come into Box Canyon and say: exterminate those fish.

D. Mulcahy Has the industry done a good job in protecting itself against diseases?

B. Busch Yes, it has. As I said, they have not had an introduction of a new disease in thirty years, until PKD or IHN in the last couple of years. And that did not come from us.

H. Ramsey They have certainly spread it around themselves, a lot of these disease organisms.

B. Busch In shipping fish.

L. Ray Have they spread it around any more than the state and government agencies have in other states?

D. Mulcahy I differ with that. We do not have it in every hatchery here.

B. Busch Your hatcheries are not twenty feet apart, either.

L. Ray You are not raising near the poundage.

D. Mulcahy That is exactly what we are struggling against. We do not want it in all our hatcheries. These are state regulations, not Federal regulations.

B. Busch Let me go through a couple points here. As far as the Idaho commercial industry goes, you say we do not have any regulations, that is exactly why I am sitting here today. That is why I came. I do not really care what you do with your fish in Washington or Oregon. That is your business. But when you start talking about a regional-type regulation, then I am sitting here.

D. Mulcahy Are you saying you are opposed to any kind....

B. Busch No, I did not say that. Let me tell you, let's break it down into three areas. All we are talking about is food fish in the Hager-man Valley of Idaho, not Idaho Fish and Game. They have their concerns that are separate from ours. One is to totally exclude Idaho food fish industry from any regulation. Arguments in favor of that is that it is very isolated geographically. It is in the upper end of the Columbia River Basin, but it is isolated geographically. There are no migrant stocks up there, that was taken care of many years ago. There is a dilution factor coming down, so it is hard to draw a conclusion of infection downstream. They virtually do not ship any live fish out of there, of any significance. What they do ship out, you have arguments, pro and con, for certifying through shipments, but it is an extremely small part of the industry. So it is a closed industry, other than the possibility of disease coming down in the water in the

dilution factor. It does not pose a large problem, in that regard-and there are arguments both ways. The second thing is limited regulation. They do not want to see any new diseases in there. And I think you would find them very acceptant of any regulation that could effectively protect them against introduction of any new diseases, particularly viral diseases and VHS. They would jump at it. Myxosoma they'd go along with because they do not have it. Myxosoma per se would not present such a problem if it were introduced because of the design and nature of their hatcheries. A limited regulation to protect them from new disease introduction, I think you would have fair support for it if it were handled properly. If you are talking about any regulations on diseases that currently exist there, I will tell you what you will definitely be only talking. You will be talking complete indemnification for all losses, and you could not even begin to add up what that would be. You are going to be talking a guarantee of no recurrence or reinfection and I do not think there is anybody in this room that would sign their name to that. You are going to be talking a regulation that is going to treat everybody equally, all private fish farmers, all state and federal fish hatcheries, whether they are mitigation, migratory or food fish-whatever, they would all be treated equally. That is tough to come up with, because they all have different concerns, priorities, constraints and so forth. It would have to be enforceable, and have teeth in it-pretty tough teeth. There would be individuals up there that would try to fight it. There is no way it would ever be voluntary-none whatsoever. Overall, it would be highly doubtful in my opinion if you would ever get any type of regulation to regulate any diseases presently occurring. At the best, maybe against introduction of new diseases.

D. Mulcahy At this meeting, have you heard anybody propose such a regulation?

B. Busch No, I have not. I am just giving you a breakdown of where I feel that industry would stand in regulation.

L. Ray Something that really bothers me here is that you have Washington and Oregon here, discussing these regulations, and you do not have a private representative from a private farm in Washington or Oregon here for any input. This is the reason--we were talking last night of why there was poor communication in the industry, and this is the reason. This meeting here is a good example.

J. Rohovec There has been communication with private industry in Oregon and Washington, I know especially within our state, Oregon. In a meeting several years ago, they were included, in fact they were probably fifty percent of the audience. Maybe you were there, Bob. So there was a lot of dialogue when Oregon was formulating their regulations. David Ransom, who works for Weyerhauser, was very much involved with us when we were writing those regulations.

L. Ray I believe it is imperative that you have that.

J. Rohovec I agree wholeheartedly. I would like to go back to some of the comments Bob made. I think if you would read both Washington and Oregon's regulations, and delve into them a little bit, you will undestand that they are not all that restrictive, and that they do exactly what Bob has suggested. They are written specifically to avoid the introduction of exotic pathogens that are not already endemic and they also make provision-and like I said, it is extremely nebulous-on how to treat ones that are already endemic. In Washington's, it is written into their policy what they are going to do with Columbia River fish. That is not unreasonable to me, and would not place any great constraints on a private producer in Washington.

D. Mulcahy And the reason there are not private growers from Washington and Oregon here is that the attendees were selected from groups that do have IHN virus, the subject of the meeting. The meeting is not regulation or laws governing fish disease. It is what to do with IHN. To my knowledge there are not any Washington or Oregon groups that have IHN problems.

J. Rohovec One of the reasons that they operate in coastal rivers is because of the problem.

An example of this is Weyerhauser. They had to bite a pretty big bullet when they were going to import eggs from Japan. They were going to import chum eggs which are unavailable in most places. They had identified a source in Japan. In the process of certifying these eggs, they found a filterable agent that causes CPE that had never been described. It blocked the introduction of these eggs even though we didn't know if it was a fish pathogen or not. It was just something that was new and they agreed it should not be allowed to enter the U.S.A. There was a lot of discussion about it, and the administrator within Weyerhauser had some ideas, and the pathologists had other ideas, and eventually they came to the realization that it would be a fairly foolish thing to do, to let those eggs in without knowing the nature of that agent.

L. Ray The difference is, if you exterminate one hatchery, you transfer those personnel to other hatcheries, rebuild, then come back.

D. Mulcahy We are not talking about exterminating any hatcheries.

L. Ray But in private industry, if you try and exterminate that, you wipe out the lives of a lot of people. If Box Canyon comes down with something, do you exterminate all the fish - you are talking five, six, seven million dollars. But that five million dollars is not any more to Box Canyon than a couple hundred thousand is to me or any other small grower. This is overlooked. They say we cannot pay eight million dollars to replace the fish here. But this guy out here, all he owns is a hundred thousand dollars of fish, we can wipe him out, he does not make any difference. That is not right. The other thing is, do you want a private industry in these states, and do you want private industries to grow. The answer to that in a lot of cases is no, as far as government agencies. I would like to see a private industry grow. So do not put constraints on it where it cannot grow.

D. Mulcahy I do not think it should be a totally laissez-faire situation where anything goes.

L. Ray I agree.

Proposed Viral Disease Control Policies for the Columbia River Basin

Einar Wold, Director

Columbia River Fisheries Development Program

National Marine Fisheries Service

Environmental and Technical Services Division

Portland, Oregon 97232

Fish disease control programs for anadromous fish in the Columbia River Basin have been maintained primarily through individual agency policies. Although numerous discussions have occurred regarding the need for a basin-wide fish disease control plan, no definitive interagency plan has been accepted. During its 1978 annual meeting, the Pacific Marine Fisheries Commission (PMFC) unanimously adopted a resolution by the five Compact States: Alaska, California, Idaho, Oregon, and Washington to "... call upon its member States and Federal agencies to convene a group of fish pathologists as soon as possible to consider and propose minimum standards concerning the transfer of live fish and live-fish products between or into the member States.*' Although this workshop is not the result of the 1978 Resolution by the PMFC, it has been called for the same reason -- the control of fish virus diseases in the Columbia Basin.

Any plan to achieve this will necessarily include identifying infected stocks, eliminating infected individuals from stocks, preventing the artificial movement of infected stocks, controlling water supplies and hatchery management practices to reduce horizontal modes of infection and establishing a network of information exchange. The establishment of such a plan has been stymied by biological and administrative questions. This workshop can and will provide the answers to the biological questions. It is my hope that we can also provide the guidance for answering the administrative questions as well. Although the following recommendations are directed toward virus diseases, I believe they can serve as a basis for all general control programs.

My recommendations for control of virus diseases deal with two time frames -- long term. comprehensive plans and short term crisis control. In either case it is imperative that the fishery agencies, because of the interstate movement of the migratory stocks, agree through a compact or memorandum of agreement to the established control measure. For the long term I suggest that the fishery agencies:

1. develop instructions, based on known epidemiology, modes of transmission, and range of disease, to cover the operations necessary to assure that disease control measures are properly implemented;
2. establish goals that emphasize improved implementation of disease control measures;
3. develop an automated data system to provide information reflecting disease occurrences and the degree to which disease control measures are implemented by each organizational unit; and
4. formulate cooperative agreements which clearly show lines of authority and responsibilities for program functions at each organizational level for both State and Federal personnel. It would be the individual State's responsibility to include all private aquaculture activities within the State's line of authority.

For the short term crisis control I propose that the Columbia Basin Fishery agencies formally agree to the following:

1. Salmonids or the progeny of those salmonids which have been exposed to waters outside the Columbia River Basin will not be permitted to be moved into the Columbia River watershed. Only eggs from disease-free stocks and treated with acceptable methods as developed by interagency pathologists will be allowed into the watershed.

2. Hatcheries with histories of IHN should establish spawning techniques so that separate incubation of eggs of each female is accomplishesd until viral examinations have been completed. Males and females must be examined for virus. Eggs/fry from all parents identified as IHN carriers must be destroyed. Eggs/fry/fingerlings from non-infected adults must be reared in water supplies free of IHN. If virus-free water supplies are not assured, individual incubation will not be successful. If IHN is diagnosed, a complete hatchery sterilization program, following guidelines developed by cooperating fishery agencies, must be completed before adults/eggs are reintroduced to the station.
3. No anadromous stocks produced in hatcheries with a history of IHN will be released in specific watersheds that are known to be free from IHN-infected stocks.
4. Individual hatchery sanitation/disinfection programs must be established and strictly adhered to where IHN is suspected.

In the event that infected stocks are identified in a hatchery where the water supply provides a continuing source of infection, the agencies, through consultation, have the following options:

1. Close down the hatchery and destroy infected stocks.
2. Reprogram to a non-susceptible anadromous species.
3. Establish strict hatchery sanitation program to lessen impact on existing'programs of the affected hatchery.
4. Construct adult barriers to exclude all migrating fish and eliminate all resident salmonids in water supply. This measure would be accompanied by a complete hatchery sterilization program.

It may seem that the above listed recommendations are harsh steps to take when programs are being pushed to release more and more fish. It is time, however, for hatchery operations to look at the long term resource needs and not only what the current year budget and rearing space can bear. Hatchery production must not be geared only to what numbers and pounds go out the planting tube but must take into consideration the effect the individual hatchery production will have in and on the total anadromous fish production in the Columbia River Basin.

Questions and Answers Following E. Wold's Presentation

W. Groberg Do the other members of the Artificial Production Committee go along with this, is there quite a range of opinions concerning regional fish disease guidelines or control?

E. Wold I said it was personal opinion on personal observations. I think I would say the Idaho representative would probably not agree with it. I do not know, I did not ask for APC concurrence.

L. Ray Something that in private industry has been said many times, is that one of our biggest problems with regulations is that the agency making the rules, basically Fish and Wildlife, is also the agency enforcing the rules. The Department of Agriculture would be a far better avenue of regulations and enforcement and then both of us, in private industry and in government Fish and Wildlife, can have input and fight over, back and forth, with another agency making the decision for enforcement. This would be far more receptive to the private industry and would be encouraged, especially within separate states.

W. Groberg How are disease control regulations enforced in the livestock industry?

K. Amos There in Washington, it is the Department of Agriculture. Every state has its own regulations. When I did research on our policy, I went to the Department of Veterinary Medicine, or whatever, and they all have their own inspection procedures, with a basis for which animals can be imported, what diseases will be inspected for, and what they will do. Most of them have indemnification programs too on destruction. That makes a big difference. It is a lot easier to isolate a few horses or cows than fish because of the nature of water. It is a very similar program though. I used a lot of their information for the basis for our program.

The problem is that the water empties out of hatcheries into other watersheds. And without expensive treatment methods, water becomes a vector for pathogen transmission. The source of IHN infection may not be virus coming out in the waste water, but cross-contamination in hatcheries. What we are looking at is perhaps Idaho fish or migratory fish, smolts, that become infected some way or another, coming down the Columbia and mixing with Oregon and Washington migratory salmonids.

L. Ray The private industry, and especially the private producers within the industry, we see the same problem as you do. Like you say, the Oregon producers fear IHN as badly as you

guys do. The problem we see is creating a god that has all this power. We are reaching the point where people like Clear Springs feel they are god themselves. They are big enough to combat you guys. But the small people-we are just at your mercy.

K. Amos PKD was an alarming situation, when they found it in Idaho, with the potential. consequences, although there was never any big problem that I was aware of. What kind of method would you use to draw together when there is a problem, whether Myxosoma cerebralis or VHS or some other exotic disease were to be introduced into a small or large hatchery? What method would you develop to solve the problem?

J. Rohovec Let's take VHS for an example because there are regulations concerning it. First, what would happen if an epizootic occurred in Clear Springs? Next, what if'it occurred in Leo's hatchery?

L. Ray This came up last year with PKD. We had several meetings within the private industry, with the state agencies, as to what should be the appropriate method. The handwriting was clear on the wall. My hatchery right across the fence from the state, if it comes down on mine, I really should exterminate, but if it happens at a seven or ten million dollar hatchery, we will have an in-house decision on what to do. This is the major problem. Who has the political clout to get done what he wants?

D. Mulcahy A lot of this is the tendency, we have to wait till something happens to decide what we will do.

L. Ray It is even worse than that. We make a regulation, but the final decision, the enforcement, waits till something happens to decide what to do.

J. Rohovec Supposedly with VHS there are regulations. If it is ever found in any hatchery, you eradicate the stock.

L. Ray Is that Federal?

J. Rohovec I think it is.

D. Mulcahy It is an agency policy, it is not a Federal law that if it is your hatchery we will come in. I think what John is asking is what would happen if it were to show up in a private hatchery in the Magic Valley? What would you do to police yourselves?

L. Ray That question was never answered last year with the PKD. If it is at my hatchery, we exterminate. If it is at your hatchery, we wait and see.

B. Busch OK. You have to look at a few things. First, let's be realistic. What are the odds of VHS showing up there, given the fact that we do not haul around live fish and so forth. If it did show up, 'where did it come from? How did it get in there? That is going to be a consideration, where it came from. What is the potential for re-exposure from that same source? That is the first question we are going to ask in making a decision. The second is, what potential harm does it have for us? With VHS, it is tremendous. Third, if we destroy the stocks, what is the potential for reintroduction again? If we can answer those questions by saying it came in by shear accident, and the route it came in we feel we can prevent from re-exposure, if we can say it is harmful and poses a serious threat to our operations, and if we can say we can destroy it, effectively disinfect it, and if we stand a good chance of not being re-exposed to it, there is no question in my mind that those stocks would be destroyed. And that is talking about biting a seven to ten million dollar bullet. And I think they would do it. But if you cannot give a positive answer to the questions, then I do not think that decision will be that easy.

L. Ray What Bob has said is the decision and policy set forth by Clear Springs, not Percy Green or Earl Hardy or the entire Valley. Each individual is going to sit and wait and make up his own mind.

W. Groberg Those are precisely the questions we (ODPW) ask each time we have had a new isolation. If you cannot appropriately answer all those questions, there is no point in destroying the fish or eggs.

B. Busch Private industry is no different. The only difference is when they bite the bullet, it is their own bullet.

J. Rohovec You keep talking about the value of the trout industry in the Hagerman Valley and I agree it is sizeable, and contributes to the economy and hires a lot of people. You also have to look at the Columbia River resource. In one twelveahour period, the spring chinook gillnet take quite a number of fish. The resource of the Columbia River is sizeable too. I do not have figures for the number of pounds of fish and numbers of dollars earned, but I would imagine it would make the Hagerman Valley look like a drop in the bucket.

B. Busch Not a drop in the bucket John, but I agree with you.

J. Rohovec If you add the recreational value of it?

E. Wold — The figure I recall for the value of Columbia River anadromous fish coming out of the basin, is $184 million per year, including recreational. If Bob is talking about $1.65 per pound, that would be $57 million per year.

B. Busch — There is no question that that is a valid consideration and I agree with Einar and his concern, and from what we have learned, and our experience with IHN and brood stock. It looks tough for migrant stocks in the Columbia River. I am glad I am raising trout. I would hate to be in your shoes.

G. Taylor — In the past two years we are seeing an upsurge in IHN in the upper Columbia River. For the first time since 1969 when they had sockeye and we had IHN there, for the first time since then, we have found IHN in spring chinook recoveries, in adults coming back. About sixty percent of the samples we have checked are positive. This scares me.

. B. Busch — I think it is the tip of an iceberg.

D. Mulcahy — That is why we are having this meeting. That is a consensus. It is bad now, but what is it going to be like in five years.

G. Taylor — In addition, that is the general area we are seeing IHN in smolts, two years in a row. In 1974 we also saw it in rainbow trout that had never been exposed to it. It came from A-l sources, and they were on spring water, so I think it's horizontal there. As far as Leavenworth is concerned, I do not know what is going on there. Potentially, we have-well, there is endemic IHN in the general watershed, and sockeye salmon in there. I think we are at the crossroads right now. The eggs are incubating right now.

L. Ray — Yesterday we said we were having higher occurrences of these viruses, IHN, recurring in the Alaska stock. If you look at it in one way, this would be expected, in that if in a wild population we have a twenty percent virus occurrence, and if we put in a hatchery to increase survival, and we release fish with an eighty percent survival carrying the virus, the other twenty percent would have died in the wild environment that we are putting into the hatchery to get a higher survival rate. So we are releasing these fish into the environment and instead of taking a twenty percent survival rate we are taking an eighty percent survival rate. This means our occurrence of the virus is going up to eighty percent. These same fish returning every year to the same hatchery, you are reproducing and breeding a higher frequency every year. We could be, by just building more hatcheries and doing more production, could be increasing the frequency of occurrence in that increased population. The more we do to clean up, the more we breed.

D. Mulcahy That is a major concern of mine that needs to be documented. Are we increasing the incidence?

L. Ray We are saving the industry by building hatcheries, but we are killing it by reproducing fish that are infected, and there is no other alternative.

D. Mulcahy That is why we are trying to figure out what to do with infected fish now. We keep them alive, we know they are infected, we turn them out now, and in five years they come back. Have we created a worse problem?

L. Ray Some of these Alaskan populations are one hundred percent infected.

W. Groberg That is where a long-term program has to come in.

D. Mulcahy That is why I am not displeased to see the State of Alaska stop its hatchery construction program on sockeye salmon. I am pleased for the fish - we do not have that choice now on the Columbia River. If we eliminate the hatcheries, it is half the catch and the Columbia River fish from the hatcheries. You want to eliminate the problem? Knock down all the dams. Given we cannot do that... You have hit on a major point that needs long-term research. If we are partially successful by keeping fish alive, are we dooming ourselves to greater problems in the future? That is the difference between a short and long term approach. You have to have both.

L. Ray I am not opposed to regulations, extermination and control. Of all the people in Idaho, I would probably favor extermination programs stronger than anyone. But my fear is that we're doing something we really do not know what we are doing yet.

W. Groberg I think all would agree that IHN is a lethal pathogen to fish. You can take a seventy or eighty percent loss, but...

L. Ray But I am not sure that the IHN we are looking at is the same IHN everyone has been talking about for the last ten years.

D. Mulcahy Could somebody in Idaho get us some isolates to look at?

B. Busch I already spoke to JoAnn. We have it in five, ten and fifty gallon drum size.

W. Groberg What you say is true, Leo. We need to know more about the strains before we can make appropriate management decisions.

J. Leong I am funded by BPA to do this.

L. Ray I would sure like to see some of the fifteen to twenty million dollars for hatcheries spent on research instead.

T. Barila The Council has put large scale hatchery production as a lower priority until some of the problems are resolved.

L. Ray It is sickening to see a hatchery raising half a million pounds of fish a year that is worth half a million dollars, torn out, and fifteen to twenty million spent to rebuild to raise three hundred thousand pounds a year.

If you can exert influence within your agencies, to combat that. The trout industry exerted a lot of influence a year ago, but people in the Corps got their toes stepped on pretty hard. You will see a lot more of that.

Too many times the government here casts their programs in concrete, in five to ten year plans. If in three years there is no IHN problem, they keep right on with their cast-in-concrete plan.

The private industries are worse than that. We asked Ken Wolf if we could get some assistance, since the private industries did not have the expertise. He said budgets were scheduled two and three years in advance, and to change anything would take an act of God. In private industry, we think the hardest thing is to stop it.

D. Mulcahy I think our agencies have to decide that IHN is a problem worth involvement until it is licked, or until we see it is hopeless.

L. Kay Personally, there is no doubt that IHN is our biggest problem production wise. However, this changes on a year to year basis and you cannot change research as quickly as problems change. This is a problem that I have seen in the catfish industry between the research community and the commercial industry. As a stabilizing force they should work together. They have a good balance and relationship.

Fish Disease Research: BPA Funding Guidelines

Theresa Y. Barila

Bonneville Power Administration

Division of Fish and Wildlife

P.O. Box 3621

Portland, Oregon 97208

I. BPA's Fish and Wildlife Funding Program

In November 1976, BPA entered into a Memorandum of Understanding with the Pacific Northwest states and Columbia River Treaty Indian tribes. The M.O.U. established BPA's funding involvement in efforts aimed at the restoration of Columbia River salmon and steelhead populations. At that time, funding was dedicated to anadromous fish research projects only.

The Pacific Northwest Electric Power Planning and Conservation Act (Regional Act), passed by Congress in 1980, expanded BPA's role and authority to include activities that protect, mitigate, and enhance fish and wildlife resources affected by the development and operation of hydroelectric projects on the Columbia River and its tributaries.

Under the mandate of Section 4(h) of the Regional Act, the Northwest Power Planning Council (Council) was to develop a Fish and Wildlife Program. Adoption was to be within one year of initial solicitation for fish and wildlife recommendations, which occured on November 15, 1981. BPA's Administrator is authorized in Section 4(h)(lO)(A) of the Regional Act to "use the fund and the authorities available to the Administrator under this Act and other laws administered by the Administrator to protect, mitigate, and enhance fish and wildlife to the extent affected by the development and operation of any hydroelectric project of the Columbia River and its tributaries". The fund is to be used to implement measures that are consistent with the Council's Fish and Wildlife Program and protection, mitigation and enhancement of fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries.

On September 16, 1982 the Council approved a draft of the program, making it available for public review and comment. As drafted, the major objectives identified in the Council's Program are: juvenile and adult migrant survival, natural and afiificial production, resident fish protection and restoration, and protection, mitigation and enhancement of wildlife and related habitat.

To highlight the'artificial production objective, our area of focus today, the Council's draft program emphasizes the necessity for additional research "to improve artificial production technology because hatcheries are a crucial link in the restoration of the Columbia River fishery." They support the recormnended concepts of low-capital salmon production facilities and tributary release of selected hatchery-reared stocks to supplement natural production. The Council also emphasizes the efficient use of facilities already available, and development of the best methods for integrating natural and artificial production. Measures to improve hatchery efficiency and quality, and those intended to provide further information on the potential contribution of recommended measures to restore salmon and steelhead runs, are given priority in the program. Recommendations requiring major capital investments, such'as the construction of new hatcheries, are deferred, pending the development of adequate controls on ocean and in-river harvest of salmon stocks.

The Council has stressed the need for a reasoned, systematic approach to developing the full potential of hatchery production, but has not identified a specific approach in its draft program. The selection of a panel of experts, knowledgeable and experienced in the areas of salmon and trout reproductive requirements, in hatchery-related biology and genetic problems, in hatchery engineering, and in water system design and engineering, has been identified in the draft program. This group would be responsible for the development of detailed artificial production objectives and criteria; the listing of potential hatchery sites based on detailed objectives and criteria; coordination of activities with a natural production team established by the Council to insure integration of natural and artificial production objectives; and review of all artificial production measures for consistency with the Salmon and Steelhead Conservation and Enhancement Act.

If this portion of the draft program is adopted, major input will be required from all the fishery agencies and entities involved in artificial production within the Columbia River Basin to develop the planning needed for a systematic approach to artificial production. It will be from individuals like you and through activities like this IHN workshop that, I believe, the real planning activities will be drawn.

BPA has generally supported the objectives identified within the Council's draft program. We believe a stronger artificial production program can be realized if these objectives are achieved. We also feel strongly that a reasoned, systematic approach to developing the full potential of hatchery technology is necessary. A disease diagnostic and

control program is one component of the systemized approach BPA would like to see developed prior to jumping into a mix of projects that have no defined goal by which to measure their effectiveness.

II. Limitations on BPA's Funding Authority

The Regional Act limits BPA's funding authority to measures which protect, mitigate, and enhance fish and wildlife to the extent affected by the development and opertion of any hydroelectric project of the Columbia River and its tributaries.

The Regional Act also cautions the Administrator that his expenditures "shall be in addition to, not in lieu of, other expenditures authorized or required from other entities under other agreements or provision of law." The plain reading of this provision shows that BPA was not intended to become the sole funding source for fish and wildlife programs.

III. BPA Guidelines Regarding Funding Disease Research

To a large extent,BPA must rely on the Region's fish and wildlife agencies and Indian tribes and, to a lesser extent, universities and others, to take the initiative in proposing research and other measures for funding. BPA has issued a "Notice of Program Interest")NOPI) soliciting proposals which seek to protect, 'mitigate, and enhance fish and wildlife resources affected by hydroelectric development and operation. The NOPI was issued to put BPA in a position to be able to implement the Council's program in a timely manner.

BPA has developed evaluation criteria to assist our selection of proposals for funding support. The most significant of these criteria is the requirement for consistency with the Fish and Wildlife Program.

IV. Criteria and Evaluation

Proposals must meet certain criteria before they can be considered for funding. This will involve evaluation of proposals against the following threshold questions:

Is the proposed project applicable to, and does it seek to resolve a problem caused by the development and operation of hydroelectric generating facilities on the Columbia River and its tributaries?

Would the funding support of the proposed project be in addition to, not in lieu of, other expenditures authorized or required from other entities, i.e., fish and wildlife agencies, Indian tribes, river operating agencies, etc.?

Is the proposal consistent with the legal rights of the region's Indian tribes?

Have the short and long-term environmental impacts of the proposed project, if any, been addressed?

Has the proposal received the endorsement of the person authorized to contract, i.e., agency Director, Tribal Chairman, University Department Head or other appropriate authority?

After the initial eligibility screening, proposals will be evaluated in accordance with the following criteria:

Contribution the project will make towards achieving the objectives of the Council's Fish and Wildlife Program;

Extent to which the project identifies an immediate problem, or problems, and provides for near-term and/or long term solutions to such problems;

The extent to which the proposed project is consistent and has been coordinated with other projects sponsored by the region's fish and wildlife agencies, Indian tribes and water managers (i.e., Corps of Engineers, Bureau of Reclamation, Public Utility Districts.)

Abilities of the applicant to undertake and complete the proposed project and adequate time has been allocated to complete the project; and

The project cost.

BPA will encourage and financially support continued planning that leads to more cohesive and directed research, well designed mitigation and enhancement measures, and an improved capability to forecast funding requirements. BPA understand6 and agrees with the importance of early implementation of measures that will immediately aid fish and wildlife. However, artificial production - specifically disease diagnostics and

control - is a specific area which, prior to initiating additional work, could benefit substantially from a focused planning effort resulting in a five-year or longer directed research and implementation plan.

Priority Research Needs Concerning Fish Viruses Prevalent Among Columbia River Basin Salmonids [1]

W. J. Groberg, Jr.

Oregon Department of Fish and Wildlife
Department of Microbiology
Oregon State University
Corvallis, Oregon 9733103804

1 Oregon Agriculture Experiment Station Technical Paper No. 6731

The Workshop "Viral Diseases of Salmonid Fish in the Columbia River Basin", brought together fisheries personnel and scientists actively engaged in a study of the IHN problem in the Columbia River basin (CRb). Part of their purpose was to formulate a document outlining the immediate research needs concerning the viruses of fish in the CRb. This paper outlines those needs (Table 1) and describes the rationale behind this outline.

I. Epidemiology of infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV).

Before a rational approach for control of a infectious agent can be attempted, the geographical distribution and incidence of the pathogen in host populations must be known. Another benefit from this data would be the identification of virus-free stocks of fish for propagation. Such data are lacking among stocks and populations of hatchery and wild fish in the CRb. Systematic surveys of these populations, using accepted and consistent methods, should be implemented and a means established to collect, store, analyze and disseminate these data.

An important part of this epidemiological study would be comparisons of IHNV isolates from throughout the CRb. Field observations and recent laboratory studies indicate that at least two distinct strains of IHNV are widespread in the basin. The existence of one strain has been known since the 1950's when it caused substantial losses in hatchery populations of sockeye salmon. less emphasis on rearing of this species resulted in a concomitant decrease in losses to IHNV among all species of fish reared in basin hatcheries. It appears that a second strain, recently prevalent in the CRb and virulent for rainbow and steelhead trout, has suddenly become established in numerous stocks of salmonid fish. Analysis of IHNV isolates would allow an assessment of the distribution and host

specificity of predominant strains identified. Analyses of strains should include, but not be limited to, studies of the electrophoretic properties of virion proteins and *in vivo* virulence tests with sockeye (kokanee) and chinook salmon and rainbow (steelhead) and cutthroat trout.

II. Pathogenesis of infectious hematopoietic necrosis (IHN).

Very limited experimental data is available that precisely documents two important presumptions that are made regarding the host-pathogen interaction between IHNV and- susceptible hosts. First, it is assumed that some proportion of fish exposed to the virus are nonlethally infected and become lifelong carriers. Generally, the virus cannot be detected in fish between the time they are young (<1.0 gram) and when they become sexually mature adults. These observations suggest that such adult carriers represent survivors of infections established when they were juveniles. Because of this assumption groups of fish, from which IHNV has been isolated, have frequently been destroyed to eliminate them as potential carrier adults. Other evidence, however, indicates that the presence of the virus in some adults is the result of a primary infection during their freshwater, adult life stage. Since the destruction of millions of young fish has been considered as a measure to reduce the incidence of carriers, it would seem prudent to conclusively demonstrate that the carrier state indeed exists prior to the further destruction of presumed carriers.

The presence of IHNV in the tissues and gonadal fluids of adult fish lends credence to a second assumption; the virus is transmitted in or on the egg and as a result the progeny from a carrier parent can be infected via the sex products (vertical transmission). This hypothesis also has limited experimental basis and depends primarily on observations made where the progeny of known infected adult populations have undergone loss

to IHNV. Millions of eggs from infected stocks have been destroyed because of this assumption. A critical evaluation of IHN outbreaks, however, reveals that many of these epizootics may be attributed to transmission of virus to young fish from carrier fish via the water utilized for egg incubation and early rearing (waterborne or horizontal transmission). As with the role of the carrier state for maintenance of virus in populations, unequivocal evidence should be established for vertical transmission of IHNV because eggs have been and will continue to be destroyed because of this concept. If vertical transmission is not a primary factor contributing to hatchery epizootics, egg destruction as a method for control would have no merit.

A third aspect of pathogenesis deserving attention addresses the recent trend of larger fish dying from IHN. Historically, IHN has been a disease of CRb fish less than one gram in weight. Recent reports from throughout the basin indicate a trend towards occurrences of the virus in yearling, and in some cases even older, rainbow trout and chinook salmon. If this is a result of the introduction of a new strain of IHNV, the value of fish lost would become significantly greater and control procedures could be dramatically affected. Laboratory experiments designed to test the susceptibioity of different ages of fish to predominant strains of IHNV are needed to elucidate these possibilities,

III. Methods for control of IHN

Because control measures for IHN are needed immediately, most basic scientific investigations will not satisfy this need. Sanitary practices can be employed immediately to reduce the impact of IHN. These practices may even prove adequate as long-term and effective methods for controlling and eliminating IHNV from stocks of fish at some locations. Egg

disinfection and rearing of eggs and young fish in virus-free water may provide both immediate and long-term control methods. Evaluation of these procedures should be conducted in controlled labor&tory conditions and should also be implemented as production tests at hatcheries. At most hatcheries the goal of a virus-free water supply can only be achieved through installation of equipment to sterilize existing sources of water. Ultraviolet light, ozonation and chlorination are effective methods of sterilizing water. The conditions at a particular location will probably dictate which method would be most suitable.

The concept of vertical transmission has led to the development of a labor-intensive, costly and inefficient procedure for spawning and rearing fish at certain hatcheries where IHNV infected stocks must be used as brood stock. These methods evolved because no alternative was available to attempt control of the disease in young fish. This procedure consists of spawning single (or few) males and females as mating pairs, sampling each parent for virus, incubating egg lots from mating pairs as discrete units (a separate water supply for each lot) and discarding egg lots in which a parent was identified as an IHNV carrier. Presumably, this technique eliminates those progeny that would have been infected by the vertical route and significantly reduces exposure and prevents infection in the progeny that came from virus-free parents. This technique will be effective only if vertical transmission is an important route for infection, if virus-free water is used for rearing, and if methods for detecting virus in adult fish are reliable. To date, no experimental or production level evaluation of the efficacy of this approach has been documented. This is primarily because production tests have not been underway long enough to properly evaluate them and where experiments have

been done, they were not broad enough in scope to provide conclusive data. Those agencies and hatchery personnel that implement this program must realize that more extensive tests must be made and they should understand the requirements for its effective use.

A significant step towards control and prevention of IHN at a facility may be immediately realized through a deliberate, in-depth evaluation of hatchery practices, its brood stocks and its water supply. Infectious hematopoietic necrosis is a contagious disease and no research is required to prove that water is a vehicle for introducing the infectious agent into hatchery fish. The presence or absence of IHN in brood fish at individual hatcheries should be determined. Thus, the presence of carrier fish in the hatchery water supply can be predicted and where appropriate, fish barriers or water sterilization equipment should be installed. Iodophore treatment of eggs and conventional sanitary procedures at a facility may help to reduce the impact or prevent the introduction of IHNV in hatchery fish.

xv. Develop a rapid diagnostic method for detection of IHNV in asymptomatic fish.

Conventional methods for diagnosis of fish viruses are frequently not rapid enough to identify carrier adults prior to the time their eggs hatch. Rapid diagnosis is imperative for the control procedure in which eggs from individual mating pairs are destroyed if either parent is a virus carrier. Since most hatcheries are not equipped to hold individual groups of fish in separate' rearing containers, the decision to destroy virus infected eggs must be made early. Additionally, a rapid diagnostic method for detecting IHNV in asymptomatic and symptomatic fish would be valuable because management decisions concerning disposition of

contaminated eggs or infected fish could be expedited. By current methods, these decisions are often delayed until a confirmatory diagnosis of IHNV is made. There are several sensitive methods for detection of antigenic material in animal tissues and these should be tested. However, before another method is adopted, its sensitivity for detection of IHNV must be compared to current cell culture techniques.

The recommended research and investigations cited are those that seem most timely to consider for the immediate problem of IHNV in the CRb. There are numerous other research projects more long-term in nature that may be required to control and prevent IHN effectively. Vacacines will undoubtedly have a role and some investigations are in progress. In-depth studies on the pathogenesis of IHNV and the mechanism(s) of transmission and maintenance of the virus in fish populations are needed. Perhaps drug or chemical therapy will also be found to have application.

The research, tests and investigations outlined in this paper represent those immediate needs for the CRb agreed upon by a consensus of fish pathologists from every governmental agency responsible for fish propagataion in Idaho, Washington and Oregon. Representatives from the commercial trout industry in Idaho also had an opportunity to participate in evaluating these needs. This work is needed immediately to curtail the spread and catastrophic losses of CRb salmonids to IHNV. Hopefully, the cooperation and efforts of these agencies will be recognized by appropriate funding sources.

Table 1. Priority research needs concerning fish viruses in Columbia River basin salmonids.

I. Epidemiology of infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV).

A. Develop a system to collect, store, analyze and disseminate data on fish viruses in the basin.

B. Survey of fish stocks throughout the basin to evaluate the incidence and distribution of both agents.

c. Define accepted and consistent methods by which viral surveys and examinations will be conducted.

D. Compare IHNV isolates from throughout the basin to determine if significant strain variation exists. Comparisons should be based at least on electrophoretic properties of viral proteins, plaque characteristics and virulence.

II. Pathogenesis of Infectious hematopoietic necrosis (IHN).

A. Determine if survivors of epizootics become carriers of the virus.

B. Determine if vertical transmission of virus from parent to egg occurs and the role of vertical transmission in epizootics.

c. Evaluate susceptibility to infection versus fish age in susceptible species (sockeye and kokanee salmon, rainbow, steelhead and cutthroat trout).

III. Methods for control of IHN

A. Laboratory experiments.

1. Evaluate the use of iodophors for egg disinfection to reduce the potential for both vertical and horizonbal transmission of virus.

2. Evaluate the use of pathogen-free water for egg incubation and early rearing to reduce the impact of the virus.

B. Production tests at hatcheries.

1. Determine if eliminating eggs fertilized or derived from IHNV positive parents can reduce or prevent outbreaks of IHN.

2. Determine if the use of iodophors for egg disinfection can reduce or prevent outbreaks of IHN.

3. Determine if pathogen free water for egg incubation and early rearing can reduce the impact of the virus.

4. Evaluate each hatchery at which IHN is a problem, for the potential for vertical and horizontal transmission of IHNV. Make recommendations on how losses might be reduced by'changing hatchery practices.

v. Develop a rapid diagnostic method for detection of IHNV in asymptomatic fish.

Questions and Answers Following W. Groberg's Presentation

D. Mulcahy On a longer term level, I think we need monitoring of the status of the disease in the river, not just mortality, but infection rate, titer distribution, infection rate in wild populations near a hatchery compared with the hatchery population; are infected fish being released now increasing the infection rate when they come back? We have a chance to get baseline information on these populations before those fish we are cutting free now come back. You have got to do those things because when they come back it is not the time to start asking if it has changed. Quantitative monitoring of the populations is needed.

J. Rohovec That is another part of epidemiology and continued surveillance.

D. Mulcahy I do not see the quantitation being done. It will take a manpower commitment.

W. Groberg I certainly can not be plaquing and quantitating without additional funds and manpower.

D. Mulcahy But we can. We can handle another thousand samples, ten locations at one hundred samples each. We'will quantitate them and send them back to you.

W. Groberg There is a logistics problem with that.

D. Mulcahy What is the alternative? You have to ask yourself if that information is worth collecting.

W. Groberg Probably at selected locations.

G. Taylor A centralized lab would be appropriate.

D. Mulcahy By discussing these things, it will be clear who should be doing what, so there is no duplicative work. I do not have a high powered basic research lab, but we do have high volume. So some of these population studies I feel would best be done in my lab; strain comparisons would be done in JoAnn's. Those are pretty obvious as to how they break down.

W. Groberg On this strain comparison, we have to have more than the electrophoresis data. In-vivo work needs to be done there and the lab at Corvallis has the capability to do it. I think there is expertise out there, with established researchers, and with new young ones; there are good people in the basin.

J. Leong The question "Are there any carriers in IHN" has been asked for a long time now. Instead of looking for the virus, maybe we should look for evidence of the virus, for example, this neutralizing factor, or inhibitory factor.

J. Rohovec They have already said that that goes away very quickly.

J. Leong The only way to look for it is for neutralizing virus. The fish serum is extremely toxic to the cells and you have to dilute it quite a bit before you put it on the cells with the virus. There are other techniques available in which you can look for a virus-binding phenomenon in serum, or cellular immunity. You cannot just go look at survivors, put them in pathogen-free water and wait a year to see if they come down with it.

D. Mulcahy That has been done with mixed results. Bob and Don Amend have both talked about this with rainbow trout. I do not believe it has been done with salmon. However, there are enough shipments of eggs and fish around into hatcheries, especially back East or into free hatcheries, where it breaks, that I believe it has to be coming in with the eggs. I am confident of the carrier state and its role in transmission, and less confident of the efficiency of transfer from a female to her progeny. Are all eggs infected, or is one in ten thousand infected? Does every female who has virus transmit it to her progeny, or is it so inefficient that one of fifty does? Is it related to the amount of virus she carries? Will progeny from a high titer fish become a high titer fish?

J. Leong But that is a separate question. What I am asking is, those fish that survive an epizootic, do they become carriers? Not vertical transmission. That is very important for your cull-out. Let's say in a particular epizootic only fifty percent die. You raise those, and they are not carriers. That is important to know.

D. Mulcahy Where is it coming from if they are not carriers? There is no known reservoir for salmonid fishes. I thought we had it on snails. We have ground everything up from slime off rocks to snails, fish, algae, frogs, anything you can catch in the water supply. There has never been a demonstration of anything except fish. If you do not accept a carrier state, you are supposing the virus persists in the environment from one year to the next. I think if you are in doubt about that you are making an error in logic.

D. Mulcahy You only find what you look for and that you have the techniques for. That is what JoAnn's pointing out.

K. Amos To determine transmission methods right now you have to use your best guess and common sense. How many resources are you going to put in to try to find that "factor"? Do you have a feeling for transmission methods, knowing what you know about IHN?

W. Groberg We have probably hatched ten thousand steelhead and kokanee eggs taken from positive adults and reared the progeny in pathogen-free water at the lab at OSU. We have sampled eggs throughout the egg development stage. We have held the fish to two years old. We have never seen IHN in any of those.

D. Mulcahy I have been chasing this question of carrier state for three years now, thousands and thousands of fish. You are right, I cannot guarantee you the carrier state exists by quantitative data. I hesitate to question it too strongly. I do not think we should base too much of our actions on the fact that it has not been demonstrated by data. I would like JoAnn to come along with DNA binding assay and look at the tissues. All we are looking for is virulent whole virions. I can tell you they are not there. It does not mean it's not part of their chromosome. We just have not looked at it in the right way.

W. Groberg Ron found it in a non-mature adult kokanee. Maybe it is there as an intact virion. Maybe it is the sensitivity involved.

D. Mulcahy What is the reservoir of infection if it is not fish?

W. Groberg Oh I think it is in the fish. In most of these observations, there are fish above that are potentially infected. Look at Cowlitz this year. No loss till seven days after they started using river water. What is incubation in the lab? Seven days. They broke a little earlier when we transported them from Kooskia.

D. Mulcahy But not every pond broke at Cowlitz after being placed on river water.

W. Groberg They all never do.

D. Mulcahy But if it's coming in the water, why don't we see it uniformly?

W. Groberg Why didn't one hundredpercent die when I put that moribund fish'in?

D. Mulcahy It is not the proportion of fish within a pond, it's the proportion of pond within a hatchery.

K. Amos Even if ponds have similar loadings, differences between ponds occur and stress factors in ponds can be different. Changing B-rated hatcheries to A-rated hatcheries, something like that is a long-term program for trying to improve the disease status of Columbia River salmonid stocks.

G. Taylor The original premise of the classification system was to improve the classifications. *This* has not happened.

J. Rohovec One way to improve it is to try to dilute out the virus by not contributing to the situation. A cull-out program is one way to do that, another is not to rear survivors of epizootics which may or may not be carriers, we do not know. Anything that would contribute to the pathogen load of the whole Basin is not going to improve it. Anything to reduce the number of infections or infectious agents will be helpful. That is happening in the B hatcheries - they get worse because they become dumping grounds for more of the same thing.

W. Groberg When we automatically terminate ponds of fish because they survived *an* epizootic, I have problems with that.

D. Mulcahy I do too, but not for that reason. It's not because they are carriers or not. The question is whether we can eliminate it from a hatchery by doing that. You have to do that the first time you find it. Once it is established in a run, the killing of infected fish will not eliminate it.

W. Groberg At Round Butte we destroyed a couple hundred thousand that survived an epizootic. I go out and look at the ponds and ask, *are* they really latently infected? I take them to the lab and cannot find virus in them. You have to know the answer before you just pull the plug and bury them. After ten or fifteen years you deserve to convince yourself that its a fact or not.

D. Mulcahy I would move the rapid diagnostic disease up, because if culling works, how will we do it? We cannot do plaquing or culture, it has to be automated and done in a couple of hours. The only thing I see is an ELISA test. If we have positive results this year with our present cull-out experiment, next year the pressure will be on for fifty thousand samples.

W. Groberg Are we going to accept the rapid technique on potentialy carrier fish without cell culture?

D. Mulcahy For the purposes of a cull-out, yes. It would have to be compared to current methods of who wanted *to* use if for certification test.

W. Groberg I got the feeling from you and JoAnn yesterday that ELISA may not be it.

J. Rohovec They were both talking about ELISA, but JoAnn was using peroxidase.

D. Mulcahy Isn't this a role of state diagnostic labs? Should BPA be funding an existing lab that is already doing survey work? Wouldn't it be better to push for agency support for their own diagnostic work?

T. Barila We cannot support “in lieu of" work. If you already have state support of say twenty thousand dollars, BPA cannot fund that first twenty thousand. We could expand perhaps.

J. Rohovec That is what Warren said. He's doing all he can and cannot do anymore. .

K. Amos There are not any more funds available in the state, yet. The responsibility for routine viral certification resides with the state, but I do not know how to do it. It should be in-house, but if you need more people. . .

D. Mulcahy Is a survey of a stock of fish for the presence of viruses, research? A survey is descriptive.

W. Groberg BPA money is not explicitly for research only. It is to enhance the resource.

D. Mulcahy How much money are we talking about?

T. Barila BPA's budget for the coming year is nine million. About one-third is committed to ongoing projects.

K. Amos The funding for hatcheries themselves is not just state. It is by Federal funds also.

D. Mulcahy BPA feels it is the states responsibility?

T. Barila Yes.

E. Wold Research priorities should not be determined by how much money is available.

T. Barila We want to make sure that what money we do have is spent productively. We will be looking at cost-effectiveness, of course. How can BPA make a decision between similar proposals? Cost/benefit has to come in, as authority for the ratepayers' funds.

J. Leong That is why a vehicle for long-term studies is 5O important. Is that the Council's role?

E. Wold Hopefully.

L. Ray I think the greatest return for your money is going to be the data collection and survey. That has to be done before you do anything else. Do not limit it only to the Columbia River Basin. You may find information available from other areas that have had IHN for years. I think you have made that clear in your survey of the hatcheries of the Columbia River Basin, but your data collection is worldwide.

E. Wold The important point of what BPA funds or does not fund is a matter by which they accept peer review of proposals that come in. I hope the expertise from this table and others available can give input in what needs to be done this year and on in to the future. People not dealing with that on a day to day basis tend to select the correct words out of a proposal and fund. This group and others can be valuable in that respect. Peer review is important especially in determining what is cost-effective, on down the road.